Understanding and predicting the leading edge dynamics of invasive rusty crayfish (*Orconectes rusticus*) in the John Day River

Mathis Loïc Messager

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Committee:

Julian D. Olden, Chair

Thomas P. Quinn

Patrick C. Tobin

Joshua J. Lawler

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University of Washington

Abstract

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Mathis Loïc Messager

Chair of the Supervisory Committee:
Professor Julian D. Olden
School of Aquatic and Fishery Sciences

Continued ecological impacts of invasive species on freshwater ecosystems is one of the main challenges confronting ecologists and decision makers in conserving biodiversity and ecosystem function today. Efforts to prohibit the initial introduction of nonnative species are widely recognized to be the most cost-effective management and policy strategy. However, when aquatic invasive species become established and start spreading through the landscape, efforts to slow their proliferation remain severely limited by a lack of adequate forecasting tools and understanding of their secondary spread. My thesis aims to address these challenges by improving our understanding of and predicting the leading edge dynamics of the invasive rusty crayfish *Orconectes rusticus (*now *Faxonius rusticus*) in the John Day River (JDR) basin, a major tributary of the Columbia River in northeastern Oregon.

In Chapter 1, I demonstrate the use of a spatially explicit individual-based model to recreate the invasion history of rusty crayfish in the JDR and forecast its future distribution. This study shows that controlling the spread of invasive species is possible even after their establishment, when control efforts can be effectively allocated, and that spatially explicit

individual-based models can provide unique insight into the secondary spread of aquatic invasive species and concretely support decision makers in choosing optimal control strategies.

Chapter 2 investigates whether phenotypic differences exist between rusty crayfish individuals at the boundary of their invasion range compared to their conspecifics closer to their initial location of introduction. I show that rusty crayfish in the JDR have developed less competitive morphology and better physiological condition as they spread towards the edge of their current invasion range and feed lower in the food web in invasion front populations than in core areas. By accounting for variations in temperature, primary productivity, and macroinvertebrate biomass throughout the invasion gradient of rusty crayfish, my research suggests that low conspecific densities and natural selection by spatial sorting are the primary drivers of these phenotypic changes, which suggests that these trends are likely to grow stronger over time as rusty crayfish keep spreading.

Together, these chapters not only improve our understanding of the leading edge dynamics of aquatic invasive species such as rusty crayfish but also improve our ability control their spread and reduce their impact on invaded ecosystems.

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DEDICATION

To Yuca.

Chapter 1

Individual-based models forecast the spread and inform the management of an emerging riverine invader

Mathis L. Messager[1, 2], Julian D. Olden[1]

[1]*School of Aquatic and Fishery Sciences, University of Washington, Seattle, Washington, USA 98195*

[2]*E-mail: messamat@uw.edu*

1.1 Abstract

1. Continued ecological impacts of invasive species on freshwater ecosystems is one of the main challenges confronting ecologists and decision makers in conserving biodiversity and ecosystem function today. Efforts to slow the proliferation of aquatic invasive species are still limited by a lack of preemptive policy enforcement, funding, and adequate forecasting tools once a species is established. Tools are still needed to guide the allocation of limited resources more effectively and efficiently for this specific invasion stage.
2. Here we demonstrate the use of a spatially explicit individual-based model to recreate the invasion history of the rusty crayfish *Orconectes rusticus* in the John Day River (JDR) basin, a major tributary of the Columbia River and only known occurrence in the western United States, and forecast its future distribution. Rusty crayfish is a voracious omnivore and its spread is raising concern about potential impacts on several native salmonids of conservation interest in the JDR watershed.
3. Our model accurately reproduced historical distribution data from extensive riverscape surveys for 2005, 2010, and 2016 with a specificity and sensitivity of ~80%.
4. Leveraging this realistic model of the spread of rusty crayfish, we show that more rapid responses to the initial invasion would have resulted in an opportunity to slow the spread of rusty crayfish. Removing 50% of the crayfish population yearly starting in 2009 (four years after their discovery) would have stalled the invasion and also been cost-effective. Without a large-scale mobilization of resources, we instead predict that rusty crayfish will reach the mainstem of the Columbia River in 2025 at the latest, at which our model predicts that the rusty crayfish population will exceed 350 million individuals over more than 1100 km of river throughout the watershed and progress further into critical salmon spawning habitat.

5. *Synthesis and applications.* Our study shows that controlling the spread of invasive species is possible even after their establishment, when control efforts can be effectively allocated. We also show that spatially explicit individual-based models can provide unique insight into the secondary spread of aquatic invasive species and concretely support decision makers in choosing an optimal control strategy.

1.2 Keywords

Aquatic invasive species, Columbia River, crayfish, individual-based models, invasive species management, John Day River, Oregon, secondary spread

1.3 Introduction

Invasive species are a primary threat to global biodiversity, economies and human health (Mack *et al.*, 2000). Recent decades have witnessed considerable advancement in our understanding of invasive species ecology, yet there is a widening disparity between the growth in knowledge and the management capacity to effectively respond (Early *et al.*, 2016). Consequently, there is a fundamental need for quantitative models that are tailored to specific stages of the invasion process, relevant to particular pathways of invasion, and whose framework can be applied to similar species (Ibanez *et al.*, 2014; Lodge *et al.*, 2016). The ability to modify the complexity of these models based on management objectives, data availability, and ecological knowledge of the focal species remains a research frontier (Gallien, Münkemüller, Albert, Boulangeat, & Thuiller 2010).

Efforts to prohibit the initial introduction of nonnative species are widely recognized to be the most cost-effective management and policy strategy (Lodge *et al.*, 2016). By combining distributional data with the ecological niche requirements of nonnative species, a rich body of literature uses ecological traits to prioritize the species for which establishment is most likely (e.g. Howeth *et al.*, 2016), applies species distribution models to identify invasion proneness across landscapes (Jiménez-Valverde *et al.*, 2011) and deploys risk-analyses to determine those species likely to cause environmental and economic harm (Leung *et al.*, 2012). By contrast, once a nonnative species has established a reproducing population, actions available to decision makers narrow considerably (Lodge *et al.*, 2016). Slowing the secondary spread from the initial point of establishment and suppressing overall population numbers of the nonnative species often are the most viable options (Vander Zanden & Olden 2008; Lodge *et al.*, 2006).

Our understanding of the patterns and mechanisms of invasive species dispersal have advanced considerably in recent years (Hastings *et al.*, 2005; Arim, Abades, Neill, Lima, & Marquet 2006), including studies on movement behavior (Rubenson & Olden, 2017), determinants of spread (O'Reilly-Nugent *et al.*, 2016), time lags (Crooks, 2005), leading edge dynamics (Johnson, Liebhold, Tobin, & Bjornstad 2006), and evolution through range expansion (Shine, Brown, & Phillips 2011). Nevertheless, mechanistic models addressing the needs of decision makers that are both spatially explicit and dynamic in time are still underrepresented in invasion research (Buchadas *et al.*, 2017). Limited resources allocated to invasive species control leaves managers grappling with where, when, and how to implement management strategies to maximize their effectiveness (Drury & Rothlisberger, 2008). Ideal modeling efforts should help support collaborations between scientists and decision makers where, among many outcomes, the trade-offs among alternative actions can be evaluated. This is particularly critical when decisions regarding the intensity and spatiotemporal allocation of species control efforts are necessary (Epanchin-Niell, Haight, Berec, Kean, & Liebhold 2012). Dynamic models have demonstrated increasing utility in this respect, where users are able to predict species spread into the future and quantitatively evaluate the efficiency of control measures differentially allocated across both space and time (Travis, Harris, Park, & Bullock 2011; Buchadas *et al.*, 2017).

Here, we illustrate the application of a spatially explicit individual-based model (SEIBM) as a mechanistic and flexible approach to understand the secondary spread dynamics of a freshwater invasive species and evaluate trade-offs between control strategies implemented across heterogeneous riverscapes. SEIBMs are a bottom-up approach to simulating animal population dynamics (Grimm *et al.*, 2005) that accounts for environmental effects on the biology and individual behavior of organisms. Individual life histories are modeled through a series of events including movement, resource exploitation, reproduction, and survival that are iterated over time. Broad ecosystem processes such as population dynamics, species interactions, and dispersal are emergent properties of the modeling process (Dunning *et al.*, 1995).

Our study focuses on the secondary spread of the invasive rusty crayfish (*Orconectes rusticus*) in the John Day River (JDR), a major tributary of the Columbia River (Fig. 1.1) and the only known occurrence west of the North American continental divide (Olden, Adams, & Larson 2009). Native to the Midwestern region of the United States, rusty crayfish has invaded 25 states and provinces in North America (USGS NAS, 2017). Rusty crayfish is an invasive species of concern in the western United States because of its potential to spread rapidly, ability to reach extremely high local abundances, and polytrophic feeding habits that cause severe ecological impacts (Twardochleb, Olden, & Larson 2013). In invaded ecosystems, they have been associated with declines in macrophytes, insects, snails, crayfish, and fish populations in a wide array of systems (McCarthy, Hein, Olden, & Vander Zanden 2006; Bobeldyk & Lamberti, 2010; Olden, Vander Zanden, & Johnson 2011). In the JDR, rusty crayfish have been rapidly spreading upstream and downstream throughout the watershed since their discovery approximately 20 years ago, raising concerns that the mainstem of the Columbia River may soon be reached (Olden *et al.*, 2009; Sorenson, Bollens, & Counihan 2012). One of the largest free-flowing rivers in the United States, the JDR is of high conservation importance as it supports a variety of fish species of significant cultural and economic value, including a population of endangered spring Chinook salmon *Oncorhynchus tshawytscha* and threatened steelhead *Oncorhynchus mykiss*.

Management strategies for controlling invasive crayfish populations have mainly focused on physical trapping and removal, biological control, and biocide use, and have resulted in mixed success. Trapping is the most widespread approach as it is less expensive, non-controversial, and amenable in support of longer-term control efforts (Hein, Roth, Ives, & Vander Zanden 2006; Gherardi, Aquiloni, Diéguez-Uribeondo, & Tricarico 2011). The use of biocides is an effective approach at eradicating local populations, and several toxicants have shown promise in terms of specificity and effectiveness (Stebbing, 2016). However, the non-target effects of biocides are a concern, particularly in running water ecosystems. Biological control by introducing or promoting populations of predatory fish has also proven successful in suppressing crayfish populations (Freeman, Turnbull, Yeomans, & Bean 2010). Ultimately, the choice of control strategies requires a careful assessment of their feasibility, effectiveness, and efficiency over time.

Our study seeks to demonstrate the utility of spatially explicit, individual-based models to forecast the spread of aquatic invasive species and assess trade-offs among different watershed management strategies. First, we simulated the recent and rapid spread of rusty crayfish in the JDR watershed from the putative time and location of initial introduction to present-day, and quantified the magnitude and geography of invasion spread at the watershed scale. Second, we deployed the individual-based model to forecast the spread of rusty crayfish throughout the mainstem and tributaries of the JDR into the future at management-relevant time scales. Third, we evaluated the management efficiency of and trade-offs among population control actions that allocate variable effort levels distributed across both space and time. This retrospective approach provides new insight into the value of early detection and rapid response strategies for invasive species management, and offers a framework that is readily applicable to other species and systems.

1.4 Materials and Methods

1.4.1 Modeling approach

Spatially explicit individual-based modeling is a bottom-up approach to simulating animal population dynamics (Grimm *et al.*, 2005), where life-history and behavioral rules are defined and applied to each individual of a population, and system level dynamics emerge from the accumulation of all individual events. Here, we use HexSim, a spatially explicit individual-based modeling framework that simulates population dynamics (incorporating processes of habitat selection, movement, reproduction, and mortality of individuals) given spatial data layers and population parameters (Schumaker, 2012). Originally developed for modeling terrestrial wildlife, we are the first to apply a recent advancement in this modeling framework, an aquatic module that facilitates the modeling of populations in dendritic or networked landscapes (HexSim 4.0 available at http://www.hexsim.net/). HexSim was chosen because it is a flexible framework in which simulation models can be designed around available data, with poorly understood processes being either omitted, represented probabilistically, or explored via sensitivity analysis. HexSim relies on a sequential-event architecture, whereby the user defines, for each population under study, a sequence of life history events (e.g., mate pairing, reproduction, movement, survival) that vary spatially and are iterated at each time step over the study period. Each event can be parameterized with different levels of complexity to account for more or less details in the underlying ecological processes. This flexibility allows for matching the degree of structural realism of the model to the availability of empirical data and knowledge of ecological processes influencing the species population structure in space and time; a relevant feature for the often uncertain and highly adaptive context in which invasive species are managed.

1.4.2 Study system

Evidence suggests that rusty crayfish were first released in the JDR near the town of Mount Vernon, Oregon, by a teacher and students of a nearby school (Olden *et al.*, 2009). Distributional data for rusty crayfish are available for 2005 and 2010 (Olden *et al.*, 2009, Sorenson *et al.*, 2012); between these surveys the leading edge of the invasion advanced downstream at an estimated rate of 13.5 km/year (Sorenson *et al.*, 2012) and densities approximating 50 individuals/m^2 were observed in some areas (J.D. Olden, per. obs.). The initial model calibration sought to reproduce rusty crayfish distributions and relative density patterns observed in August 2005 and August 2010, based on a putative introduction date of June 1999 (i.e., the end of the school year when the classroom release event likely occurred).

HexSim relies on a network structure whereby the movement of each crayfish takes place at the river reach scale and therefore crayfish life history events unfold in response to environmental conditions in the particular reach. Here, the National Hydrography Dataset NHDPlus version 2 was used as the stream network basic structure (Horizon Systems Corporation, 2007). The NHDPlus v2 stream network comes with a full set of attributes for each reach, including total reach length (km), gradient (%), and modeled mean monthly and annual discharge (m^3/s). River width (m) for each reach was modeled based on downstream hydraulic geometry (Leopold & Maddock, 1953) with a log-log linear regression between mean annual discharge from the NHDPlus v2 and satellite imagery-derived estimates of river width (Appendix 1.1). The surface area (m^2) of each reach was then computed as the product of its estimated width and the NHDPlus v2 length. The literature suggests that rusty crayfish do not occupy or survive in reaches characterized by intermittent hydrology (Claussen *et al.*, 2000). Therefore, tributaries labeled as intermittent in NHDPlus v2 or with modeled August (month of minimum basin-wide average discharge) mean discharge < $7\text{x}10^{-3}$ m^3/s were omitted from the analysis. This discharge threshold was confirmed with a cursory wet-dry field survey of tributaries along the main stem of the JDR in August 2016 (results not shown). Only one potential physical barrier to dispersal, a low head dam, exists on the main stem of the JDR about 15 km upstream of the putative location of rusty crayfish introduction. We did not include this dam in the dispersal model as it appeared too small to act as a permanent barrier.

Average daily water temperatures from 1999 to 2016 were estimated for each reach following McNyset, Volk, and Jordan (2015). In brief, multiple regression models were built separately for spring and autumn of 1999 and 2000-2016 relating average daily water temperature (°C) recorded across the watershed to a suite of predictors [i.e., remotely-sensed daily land surface temperature (°C), calendar day, watershed area (km^2), and elevation (m)], for a total of four models (Appendix 1.1). The mean predicted daily water temperature in each reach from 2012-2016 (representing 5 out of the 10 hottest years over the study period) were used to simulate water temperatures from 2017 to 2025.

1.4.3 Crayfish life history

To estimate rusty crayfish population dynamics, a number of model structures and parameters using the same common life-history were evaluated based on 10 replicate runs. Rusty crayfish is a particularly well-studied species; a systematic literature review of their biology yielded 210 publications that informed the parameterization of the models (Appendix 1.2, 1.3, and 1.4). All models rely on female-only demography and are initiated by the introduction of 10 female rusty crayfish propagules between the towns of Mount Vernon and John Day in Oregon (44.416° N, 119.090° W). All crayfish in the model undergo a four age class life cycle in which a portion of the population moves and dies every month, and mating occurs yearly. Every year, a percentage of individuals in every age class produce a number of offspring drawn from a normal distribution whose mean and standard deviation depend on the parent's age and the density of crayfish in their reach. Density dependence was therefore included in the model as a linear relationship between recruitment and local crayfish density. Because the factors that can contribute to mortality are numerous and their specific influence is unknown, mortality was kept constant for all crayfish of a given age class and occurred as a stochastic process at every monthly time step.

Due to the computational requirements of the model, juvenile crayfish (age 0) were aggregated into "super-individuals", each representing four individuals. This method, commonly used in individual-based modelling (Scheffer, Baveco, DeAngelis, Rose, & van Nes 1995), allowed us to decrease the processing time associated with large and short-lived peaks in population size in the first few summer months following the birth of juveniles without compromising model performance. This scale factor of four is considered low for the population size estimated in the JDR. Based on our preliminary analysis, we observed that the effect of this approach on model predictions was consistent over time and space, and only resulted in a slight underestimation of spread.

1.4.4 Crayfish dispersal

In the HexSim aquatic module, crayfish movement was performed along a network of two-dimensional branching segments over distances stochastically drawn from a defined dispersal kernel. Often derived from mark-recapture data, a dispersal kernel is the probability of an individual moving a given distance away from its original location. In contrast to the wealth of information regarding the ecology of rusty crayfish, fewer estimates of dispersal rates exist that are specific to river systems; and rates of rusty crayfish spread observed in the John Day

River far exceed those reported in the literature (Appendix 1.4). Therefore, the monthly dispersal rate of rusty crayfish individuals for this model was determined through inverse modeling and calibration. In other words, parameter values of dispersal were adjusted to match observed crayfish distributions through time, according to principles of pattern-oriented modeling (Grimm *et al.,* 2005).

The broader crayfish literature points to the primary influence of water temperature and flow velocity on individual dispersal rates. Both of these factors were included in our SEIBM. Rusty crayfish can tolerate a wide range of conditions yet their metabolic rates depend on water temperature. Growth and survival rates are parabolic from 0°C and 40°C with a thermal optimum between 20°C and 25°C (Appendix 1.4). Therefore, although rusty crayfish can remain locally active at low temperatures, large-scale movement is rare. Prins (1968) reported a decrease in rusty crayfish activity below 6-8°C and complete torpor at 4°C, and Hamr (1997) found that rusty crayfish were immobile between 0 and 6°C. Based on these studies, the model assumed that movement distance of each crayfish was linearly dependent on the proportion of days each month when average daily water temperature exceeded 6°C. This calculation was performed at the reach scale. For example, if average daily water temperature exceeded 6°C for 15 days in a month, crayfish were moved half the distance relative to what is possible if all days exceed 6°C. If a crayfish reached a confluence where one tributary's water temperature was lower than 6°C for at least one day of the month while the other's exceeded 6°C all month, it would disperse to the warmer tributary with a probability of 0.8. This probability was chosen to account for the reported thermal preferences of rusty crayfish (Appendix 1.4).

Evidence from the literature points to a significant directional bias of crayfish dispersal in river networks, with invasions spreading faster downstream than upstream (Appendix 1.4).

Accordingly, an “upstream resistance” parameter was created that proportionally decreases the upstream distance moved by crayfish according to the slope of the river reach they are traversing. Reach slope is a reliable correlate of flow shear stress and physical barriers experienced by crayfish. Parameter values were iteratively examined to correspond to observed patterns of dispersal in the JDR.

1.4.5 Model validation

A pattern-oriented modeling approach was used to ensure that the SEIBM possessed a satisfactory accuracy for its intended application (Grimm *et al.*, 2005). We compared modeled crayfish spread throughout the JDR to known occurrences according to two comprehensive surveys. For 2005, data consisted of 10 presence and 8 absence records accompanied by qualitative assessments of relative density (Adams, 2005; Olden *et al.*, 2009; J. Adams, per. com. January 2016). For 2010, 12 presence and 10 absence observations were recorded, accompanied by semi-quantitative estimates of crayfish density (Sorenson *et al.*, 2012). The precise downstream leading edge locations could not be determined for both these times due to limited access to the river but the tapering of crayfish densities towards the downstream distributional extent limit was used as the basis for an estimation.

We assessed model performance in three ways. First, we calculated model correct classification, sensitivity (the percentage of correctly classified presences) and specificity (the percentage of correctly classified absences). Second, we compared the mean watercourse distance between the modeled and observed invasion edges. We calculated the mean and standard deviation of both sets of performance metrics over 10 runs for each model structure. In general, we applied a minimalism criterion to model development by avoiding the inclusion of complex functional relationships into the model when no data supported a specific form. Third, we assessed model performance according to the present-day distribution of rusty crayfish. Guided by the predicted distribution of rusty crayfish for August 2016, surveys were conducted at 60 sites distributed every 5-10 km on the main stem and primary tributaries of the JDR from August 1st to 22nd, 2016. Lower-order tributaries were also visited to assess their hydrologic condition and determine the occurrence of rusty crayfish. At each site, area-standardized kick-seining was performed in six locations across a 50-m long reach. One person disturbed 1 m^2 of substrate upstream of a seine net held by another team member to flush crayfish downstream, yielding a mean and standard deviation of crayfish density at each site. To ensure consistency in our measure of relative density, we exclusively sampled in runs when possible, as this mesohabitat provides the most adequate flow velocity and depth for this sampling method (Larson & Olden, 2016). To avoid false absences, we conducted three additional survey approaches when rusty crayfish were not detected using seining. First, one person snorkeled the entire survey reach for 20 minutes, visually inspecting and lifting substrate to count crayfish. Second, another person walked the streambed to search for and capture crayfish with a hand-net in coarse substrate (optimal habitat for rusty crayfish) for 20 minutes. Third, 10 cylindrical, wire-mesh crayfish traps (0.42 m long, 0.21 m diameter with two 60-mm openings) baited with ~100 ml volume of commercial crayfish bait were set overnight in pools and deeper runs. The previously described performance metrics were calculated in reference to the present-day distribution.

1.4.6 Model refinement and sensitivity analysis

Following our assessment of the accuracy of the initially chosen model, we retained its structure and used the data on the distribution of rusty crayfish in August 2016 (while still considering 2005 and 2010 distributions) to adjust the dispersal kernel of the model. Our aim was to retain the same model structure, but adjust the dispersal parameter through pattern-matching such that our model could both adequately hindcast crayfish spread from initial introduction to 2016 and capitalize on all available data to produce the most robust forecasts for 2025. Next, local sensitivity analysis was performed by varying one parameter at a time in the final model. We tested 4 different values in addition to these in the final model (+20%, -20%, -75%, and +100% of the parameter values in the final model) for the following parameters: number of initial propagules introduced at the beginning of the model, intrinsic fecundity, carrying capacity, and juvenile survival. Moreover, we tested different shapes of the dispersal kernel, influence of temperature on movement, and upstream resistance values. Sensitivity to these changes was assessed by comparing the modeled mean population size and distribution of rusty crayfish in 2005, 2010, and 2016 over 10 replicates among model structures.

1.4.7 Assessing the effectiveness of crayfish control strategies

We quantified the potential effects of control measures on the distribution, density, and rate of spread of rusty crayfish in the JDR. The most common outcome from previous crayfish control attempts is that eradication is not a feasible goal once a population of invasive crayfish is established, particularly in open systems like rivers (Freeman *et al.*, 2010; Gherardi *et al.*, 2011; Stebbing, 2016). However, control measures to slow the spread of a population and reduce overall densities (a direct determinant of ecological impacts), can be successful and desirable (Simberloff, 2009). Here, we simulated hypothetical control efforts involving mass crayfish removal through trapping occurring across the entire population range with different levels of intensity reflecting additional yearly mortality of 10%, 17.5%, 25%, 37.5%, and 50%. Moreover, we examined the effect of initiating these control strategies at different times since initial invasion: two years (2001), six years, (2005), 10 years (2009), 14 years (2013), and present-day (2017).

To evaluate whether the tested measures were realistic, we also estimated the trapping effort that would be required to achieve these levels of mortality. For each reach in the watershed, we computed the required effort E (number of trap nights) to achieve a given level of mortality M (% of crayfish in each reach) based on the population size in that reach N and the trapping catch-per-unit-effort (CPUE, crayfish caught/trap night) corresponding to the density of crayfish D (crayfish/m^2) in that reach. To define the relationship between D and CPUE, we used data from Dorn, Urgelles, and Trexler (2005) and from our field survey according to:

$$E = M*N/D^{1/1.3}$$

Assuming that crayfish would preferentially occupy optimal mesohabitat when competition is limited, we used a minimum density of 0.1 crayfish/m^2 when computing effort to account for targeted trapping by managers. This avoided unrealistically high efforts needed to achieve the required levels of trapping mortality in reaches with very low crayfish densities. We assessed the effectiveness of control measures by comparing the population size and invasion extent (length of invaded watercourse) of rusty crayfish in 2025 among the different management scenarios, and quantified trade-offs by comparing required trapping effort over time.

1.5 Results

1.5.1 Model performance and predictions

The modeled spread of rusty crayfish across the JDR Basin, from initial introduction in 1999 to distributional extent in 2005, 2010, and 2016, accurately reproduced the observed distribution of rusty crayfish over that period (Fig. 1.2). The initial model calibrated using data from 2005 and 2010 prior to our survey of crayfish distribution in 2016 yielded specificities (percentages of accurately predicted presences) of 91% (2005, n=10), 83% (2010, n=12), and 78% (2016, n=46), and sensitivities (accurately predicted absences) of 90% (2005, n=8), 80% (2010, n=10), and 69% (2016, n=8). Most unmatched absences in 2016 were located in small side tributaries where no crayfish were found during our survey. The initial calibration process showed that increased complexity in the dispersal component of the model rapidly improved the predictions compared to simpler model structures, particularly when incorporating movement asymmetry in the upstream and downstream directions.

The 2016 distribution of rusty crayfish indicated rates of downstream spread that exceeded model predictions by at least 50 river km (RK) and perhaps as much as 80 RK. Consequently, the dispersal component of our initial model was re-calibrated to account for the model tendency to under-predict the edge. We increased the probability of long distance movement in the dispersal kernel while keeping all other model parameters identical to the initial configuration (Appendix 1.5). This appreciably improved the performance, increasing the predicted downstream spread in 2016 by 49 RK. The sensitivity and specificity of the resulting model decreased for 2005 (86% and 80% respectively), but increased for 2010 (87% and 80%) and 2016 (84% and 74%) compared to the initial model. Whereas this new model overestimated rusty crayfish downstream spread in 2005, it accurately hind-casted the leading edge in 2010 and only slightly underestimated it in 2016. Upstream, the leading edge in the North Fork of the JDR was overestimated in 2010 by at least 15 RK while it was accurately predicted in 2016. Furthermore, the upstream leading edge was also accurately predicted for all years of record in the South Fork JDR and the main stem.

The HexSim model predicted, likely conservatively, that by August 2016, the population of rusty crayfish in the JDR was at least 48 million individuals (including juveniles) and occupied at least 705 km of river throughout the watershed (Figs 1.2-1.3). In both the initial and final models, crayfish densities were generally underestimated throughout the watershed in the initial years of the simulation. Modeled crayfish densities in the main stem did not exceed 0.1 crayfish/m^2 for the summer of 2005 and 1.0 crayfish/m^2 for the summer of 2010 but were within the range of observed densities in 2016 (Appendix 1.6). Moreover, core population areas with the highest densities were predicted to be further downstream than we observed in 2016. This led to an overall overestimation of crayfish densities in the North Fork JDR and an underestimation of densities in the South Fork JDR despite matching invasion extents, as crayfish disperse in these tributaries from the main stem JDR (Appendix 1.6).

Model predictions supported the high likelihood that rusty crayfish will spread and invade the mainstem of the Columbia River at least by 2025 (Figs 1.2-1.3). At that time, the crayfish population is expected to exceed 350 million individuals and extend over more than 1,100 km of river, including almost all major tributaries of the JDR. Notably, rusty crayfish are forecasted to advance beyond the confluence of the North Fork and Middle Fork upstream. The modeled invasion edge is predicted to progress by less than 10 km upstream in the South Fork and upper main stem, without even considering the low-head dam in the main stem.

1.5.2 Sensitivity analysis

The model results were robust to parameter changes within 20% of their calibrated value, particularly for the estimates of spread (Appendix 1.7). Estimates of population size were most sensitive to life history parameters such as yearly survival of juveniles and fecundity (e.g. 190% increase in estimated population size in 2016 with 20% increase in fecundity) and relatively invariant to changes in dispersal structure and parameters (Appendix 1.7).

The extent of river predicted to be occupied by rusty crayfish was sensitive to both life history parameters and dispersal parameters, though it varied the most to changes in the latter. Small changes in life history parameters, on the order of ±20%, only marginally influenced estimates of spread (< 20% difference in invasion extent for all parameters and years). Interestingly, a change in the influence of temperature on movement, from a dependence on the number of days above 6°C to a parabolic response, did not significantly influence spread (< 5% change in invasion extent). Furthermore, changes from a constant movement distance for all individuals in the population to a leptokurtic dispersal kernel with rare long distance events nearly doubled the spread of rusty crayfish, in spite of the mean movement distance remaining the same. Indirect patterns also arose. For instance, increased resistance to upstream dispersal not only reduced the amount of RK invaded by rusty crayfish but also indirectly increased the speed of downstream dispersal. In addition, increased long distance movements in the dispersal kernel led to more instability of the upstream edge and diminished progress of the population upstream.

Given that the rusty crayfish population in the JDR exhibited near-exponential growth in the first years of the simulation, increasing net population growth rates in the model led to dramatic increases in population size within a few generations. A doubling in juvenile survival led to a surge in population size in 2005 and 2010 by more than an order of magnitude compared to our final model. Yet, 2016 population levels reflected the dampening role of density dependent processes on the effect of increased survival or fecundity. In general, parameters leading to lower net population growth rates or higher carrying capacity led to increased variability in population and sometimes to extirpation in the first few years for the former. Increased population growth rates, on the contrary, decreased the importance of stochasticity, particularly when carrying capacity was quickly reached in large swaths of the watershed (e.g. in 2016).

1.5.3 Comparing management strategies for controlling invasion spread

Our analysis showed that control of rusty crayfish spread would have been both possible and cost-effective if started during the early years of its invasion (Fig. 1.4). Model simulations demonstrated that yearly removal of 25% of the rusty crayfish population starting in 2001 could have led to a 95% decline in population and a 44% decrease in invaded river kilometers (625 km) by 2025 compared to projected levels without actions (Fig. 1.5a). Initiating the same level of yearly removal effort in 2009 could have led to a 70% decline in population and a 27% decrease in invaded river kilometers (806 km) by 2025 (Fig. 1.5b). Encouragingly, our model showed that high levels of efforts (50% mortality), even if enacted relatively late (e.g. 10 years after the introduction and 4 years after the detection of rusty crayfish, in 2009) could have appreciatively slowed the spread of rusty crayfish in the JDR (Fig. 1.4c). In this scenario, the leading edge was predicted to have progressed downstream by only 95 RK between 2010 and 2025 as compared to the projected spread of 215 RK into the main stem of the Columbia River without action.

In terms of cost, our model demonstrated that early action at moderate (25% mortality) to high (50% mortality) levels of effort would have not only be most effective but also required the least overall expenditure (Fig. 1.6). By 2025, we estimate that removing 25% of the rusty crayfish population yearly starting in 2001 would have required half of the cumulative trapping effort as if starting in 2009. Nonetheless, insufficient efforts, even if implemented early, could lead to runaway increases in costs regardless of the starting year given the high fecundity of crayfish. Specifically, responding early to the invasion (in 2001) but with insufficient levels of efforts (10%) would still be less effective and more costly than more intense control (25%) undertaken much later (2017).

Attempts to control the spread of rusty crayfish starting in 2017 would be extremely challenging unless large resources were invested. Doubling control mortality would be required to achieve similar results as 25% removal efforts starting in 2001. It would result in a 90% decline in population size and constrain crayfish spread by 37% by 2025, likely preventing it from reaching the main stem of the Columbia River (Fig. 1.4d). Implementing this approach would require setting over 60,000 trap-nights every year from 2017 on (a trap night represents one trap set for 12h overnight; 1,000 trap nights can be 1,000 traps set over one night or 100 traps set over 10 separate nights).

1.6 Discussion

Using dynamic simulation modeling of rusty crayfish spread over a two-decade period and forecasting its future distribution, we demonstrated that rapid response to their detection could have prevented this quick advance and avoided potentially dramatic consequences. Enacting substantial removal efforts upfront, in addition to being more effective, would also have resulted in much lower costs of action if prolonged population control is anticipated, according to our model. Model outcomes provide direct guidance to managers grappling with the minimum effort levels required to limit both spread and cost. For example, we estimate that removing 50% of the population of rusty crayfish throughout their range yearly starting in 2009 would have largely stalled the invasion and required about 15,000 traps per year until 2024, the equivalent of 3 months per year of work for a field technician. Halving the removal rate would not prevent dramatic population growth and would thus require over 4 times more trapping by 2024. Our forecast of the future distribution of rusty crayfish can also guide management agencies in the JDR in prioritizing areas of efforts to sensitize the public on the topic and enact bans on transporting crayfish out of the watershed, an effective approach to prevent further introductions in the region (Dresser & Swanson, 2013).

The fisheries of the Columbia River are paramount to the culture and economy of the region; therefore, the rapid advance of the only known infestation of rusty crayfish in the western United States towards the river's mainstem and into salmon spawning grounds is a cause of significant concern. It appears that only a large-scale mobilization of resources will slow the current rate of spread of this invasion. However, our model simulations suggest that management efforts can still reduce overall population size. Although range containment is often a preferred management outcome, the ecological impacts of invasive species can be minimized by reducing local population densities. In the United Kingdom, five- and three-fold increases in macroinvertebrate abundance and richness, respectively, were reported when invasive signal crayfish (*Pacifastacus leniusculus*) densities were reduced following a single trapping season (Moorhouse, Poole, Evans, Bradley, & Macdonald 2014). The ability to reduce crayfish impacts via removal efforts is particularly relevant in the Columbia River where threats to Pacific salmon by rusty crayfish are possible, but still unknown. Evidence shows that invasive signal crayfish lower eggs-to-fry escapement, increase fry mortality, and decrease juvenile sheltering in Atlantic salmon *Salmo salar* (Griffiths, Collen, & Armstrong 2004; Edmonds, Riley, & Maxwell 2011). Apparent and resource competition could also be key in driving the effect of crayfish on native salmonids. Increases in abundance of predatory fish like smallmouth bass through predation on dense rusty crayfish populations are likely to interact with reduced shelter use by salmonids in challenging efforts aimed at restoring endangered and threatened salmonid populations in the JDR (Carey, Sanderson, Friesen, Barnas, & Olden 2011). Management efforts seeking to decrease overall crayfish densities could therefore be key in protecting these native species.

By using processes as the building blocks of spatial population modeling, individual-based models allow us to explore discrepancies between predictions and observed patterns to implicate potential alternative or unknown mechanisms in operation. Our model accurately reproduced historical distribution data with high specificity and sensitivity, but estimated crayfish densities were consistently underestimated compared to surveyed densities in 2016. Three reasons may explain this result. First, rusty crayfish may have been first introduced earlier than previously thought and subsequently had longer to reach higher densities in the early stages of the invasion. Second, a prolonged lag between the initial establishment and the inception of long distance dispersal events (often characteristic of secondary spread) may have occurred (Crooks, 2005). Third, depensatory predation by smallmouth bass (*Micropterus dolomieu*) and northern pikeminnow (*Ptychocheilus oregonensis*) could have slowed the initial spread of crayfish at low densities before rusty crayfish reached higher densities where predation had little effect (Baldridge & Lodge, 2013). In the absence of knowledge about the specific processes in operation, we decided not to include additional time lags into our model structure.

Our results suggest that the rusty crayfish invasion is advancing downstream at an increasing pace in the JDR. Accelerating speed of invasions are often attributed to long-distance movements, environmental heterogeneity, temporal variability in reproduction and dispersal, and evolution (Hastings *et al.*, 2005). One possible explanation for the slight underestimation of crayfish spread may be that the dispersal kernel in our model is not sufficiently leptokurtic (fat-tailed), and thus misjudges the number of extreme distance dispersal events. Higher temperatures and elevated productivity downstream could also lead to increased metabolism and fitness at the downstream edge of the invasion. However, neither relaxing the minimum temperature at which crayfish move in the model (resulting in longer annual periods of downstream movement) nor allowing for a parabolic increase in movement distances with temperature were able to better account for the observed rates of spread (Appendix 1.7). Furthermore, increases rather than decreases in the abundance of fish predators downstream are evident, and therefore do not explain the downstream acceleration of spread.

The observed acceleration of rusty crayfish invasion may stem from phenotypic shifts favoring dispersal traits at the invasion edge and/or increased passive dispersal as the edge advances downstream. A number of studies have shown that shifts in the phenotype of invasive species populations at their expanding edge could lead to the dispersal kernel changing over time, leading to greater movement (Phillips, Brown, Travis, & Shine 2008). Phenotypic shifts at the invasion edge have been observed in both terrestrial and aquatic environments (Chuang & Peterson, 2016), including several ongoing crayfish invasions (Pârvulescu, Pîrvu, Moroşan, & Zaharia 2015; Rebrina, Skejo, Lucić, & Hudina 2015). Changes in morphological (e.g. leg length), behavioral (e.g. increased boldness), physiological (e.g. faster growth rate), or life history (e.g. earlier maturation) traits at the invasion edge can arise through phenotypic plasticity (Davidson, Jennions, & Nicotra 2011) as well as natural selection (Brown, Kelehear, & Shine 2013) and spatial sorting (Shine *et al.*, 2011).The spatial sorting process involves the fastest dispersing individuals at the expanding edge of the population systematically interbreeding, and if dispersive traits are heritable, this will result in enhanced dispersal ability in their offspring.

This continues in subsequent generations until trade-offs between traits begin to limit the potential for directional selection (Burton, Phillips, & Travis 2010). In the case of rusty crayfish, it is possible that strong selection on enhanced dispersion at the invasion edge has occurred over the nearly 20 generations since initial introduction in the JDR, leading to increasing rates of spread. Several traits seem to be labile in rusty crayfish; individuals from invasive populations exhibit faster intrinsic growth rates and higher survival than those from the native range (Sargent & Lodge, 2014). Accelerating dispersal rates over time could also partly explain the prolonged lag between the establishment of the population and its rapid downstream spread. Taken together, we believe that the dispersal kernel has changed over time, suggesting that rusty crayfish will reach the Columbia River several years before the model predicted year of 2025, perhaps as early as 2020.

Crayfish spread may occur through both active dispersal (i.e. crawling) and passive drift during high flows; the latter mechanism potentially also explaining why the downstream extent of the rusty crayfish invasion was underestimated. Given that flow velocity and water depth increase downstream, the distance travelled by crayfish if dislodged by high flows would increase moving downstream, potentially leading to the observed acceleration of spread. However, despite passive dispersal being the main mode of dispersal for the majority of stream macroinvertebrates (Bilton, Freeland, & Okamura 2001), the existence or importance of passive drift for crayfish dispersal remains unknown, aside from anecdotal evidence (Robinson, Thom, & Lucas 2000) and hypotheses (Sorenson *et al.*, 2012), particularly regarding juvenile crayfish (Bubb, Thom, & Lucas 2004). In the JDR, higher numbers of crayfish in rotary screw traps during flood periods suggest that crayfish drift in response to high flow events (Keith DeHart, per. com. March 2016). Long distance passive dispersal during high flows could thus explain the unusually large distances required in our model to accurately reproduce the downstream spread of rusty crayfish, the accelerating rates of spread downstream, and the asymmetry between upstream and downstream dispersal observed in the JDR and elsewhere (Momot, 1996).

Our modeling approach seeks to bridge the management gap that arises once a nonnative species becomes established and provides strong quantitative evidence for the importance of early detection and rapid response for managing expanding populations. Even though eradication in an open system such as the JDR is unrealistic (Myers, Simberloff, Kuris, & Carey 2000), low surveillance levels can lead to the early detection of introduced populations and trigger swift responses, greatly reducing long term control and damage costs (Epanchin-Niell *et al.*, 2012). We imagine that the prioritization of surveillance across the Columbia River Basin could be guided by the combination of a broad-scale screening model to identify high-risk locations of initial introduction and a SEIBM to predict the spatiotemporal dynamics of secondary spread (Ibanez *et al.*, 2014). Accommodating variable quality of data and uneven knowledge of target species, decision-makers can leverage this predictive model to ask “what if” questions, assessing the optimal intensity and most efficient allocation of control efforts to achieve desired reductions in population levels and invasion extent. Such a framework could significantly strengthen existing efforts to promote invasive species management as a pillar of the Columbia River Basin restoration program (Naiman *et al.*, 2012). In the case of rusty crayfish, our recommended trapping effort intensity given rapid action (2001-2009) is similar to the successful five-year large-scale removal of rusty crayfish in Sparkling Lake, WI (Hein, Vander Zanden, & Magnuson 2007) and slightly inferior to that in the experimental removal of signal crayfish in the River Windrush in the U.K (Moorhouse *et al.*, 2014). Although promising, the implementation of any large-scale control action should involve a transparent evidence-based assessment of risks and uncertainty by an independent research group to avoid undesirable outcomes (Kopf *et al.*, 2017). For example, given the central role of crayfish in smallmouth bass diet, the suppression of high densities of rusty crayfish in the JDR could have temporarily negative effects on native species by shifting predation pressure onto fishes and macroinvertebrates, as has been observed in Sparkling Lake, WI (Hansen *et al.*, 2013).

Because of its intuitive and flexible design, the structure of this model can easily be communicated to stakeholders and modified by the management community with additional data and control strategies over the course of an adaptive management plan. In particular, various control methods could be combined to find the most effective strategy that fits the current stage and context of the invasion (Stebbing, 2016). Physical barriers for instance could be installed to slow or halt the upstream spread of crayfish (Dana, García-de-Lomas, González, & Ortega 2011). In the JDR, only a few individuals were found upstream of a low-head dam despite high densities observed immediately downstream. The model can thus be altered to evaluate whether demographic Allee effects upstream of barriers could be exploited by trapping to prevent further spread (Tobin, Berec, & Liebhold 2011). In another possible scenario, the cost of simultaneous trapping and biological control, as implemented in a whole-lake removal of rusty crayfish (Hein *et al.*, 2007), can be compared to a single large-scale biocide application. In an open system such as the JDR, biological control could be implemented in the model as a seasonal longitudinal change in fish predation pressure due to modified fishing rules. Most importantly, the allocation of effort towards these various approaches can be explored in space and time to best reduce crayfish densities and spread.

Our sensitivity analysis, together with a review of the literature, provided confidence regarding the model parameter estimates. By varying parameters in small (±20%) and large increments (-75%, +100%), we demonstrated that the population size projected by our model was sensitive to changes in juvenile survival and intrinsic fecundity for population size, but was largely invariant to carrying capacity or the number of propagules seeding the initial invasion. The sensitivity of the model outputs to changes in some parameters is not entirely surprising given the inherent stochasticity of biological invasions (Lewis & Pacala, 2000; Crooks, 2005), a fact also reflected in the observed variability across 10 replicate runs for a given set of parameters. However, considering that rusty crayfish is expected to reach carrying capacity in much of its range by 2025, we expect that our projections of crayfish population size are a robust first estimate.

In this study, we show that early action could have halted the projected invasion of rusty crayfish into the main stem of the Columbia River by 2025, based on an accurate model of the spread of rusty crayfish in the JDR from 1999 to 2016. Our model also suggests that considerable investment in removal efforts now could still curb the rate of spread and population size of rusty crayfish downstream. Although numerous models exist to assess those areas most at risk of invasive species introductions (Olden *et al.*, 2011), the current dearth of quantitative tools available to resource managers limits the effectiveness of control measures aimed at limiting the spread of invasive species that are already established (Vander Zanden & Olden, 2008; Ibanez *et al.*, 2014). We demonstrate here the utility of spatially explicit individual-based models to forecast the secondary spread aquatic invasive species and evaluate different management strategies aimed at population control. Beyond enhancing our current understanding of the invasion of rusty crayfish in the JDR, our work therefore demonstrates that controlling the spread of invasive species is possible even after their establishment and provides a novel way to allocate limited management efforts effectively in the fight against aquatic invasive species.

1.7 Author's contributions

M. L. M. and J. D. O. conceived and designed the study, obtained funding, collected data, interpreted the data, and prepared the manuscript. M. L. M. designed and implemented the model and analyzed the data.

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1.10 Figures

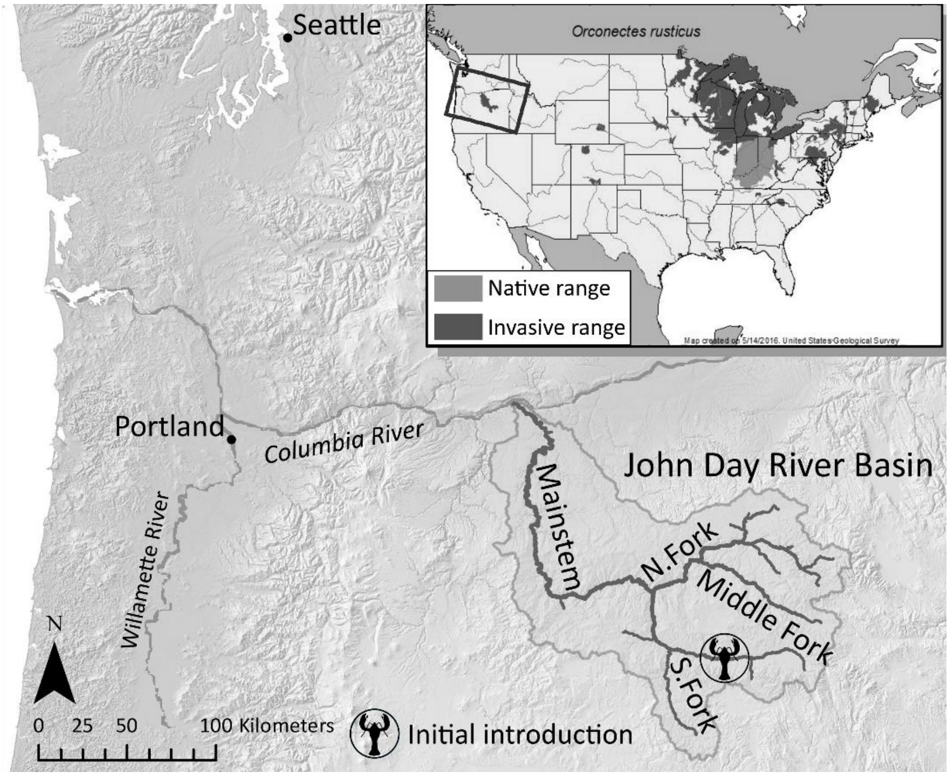

Figure 1.1. Native and introduced range of rusty crayfish *Orconectes rusticus* in the conterminous United-States (inset) and regional map of the John Day River basin with the hypothesized point of initial introduction (Olden et al., 2009).

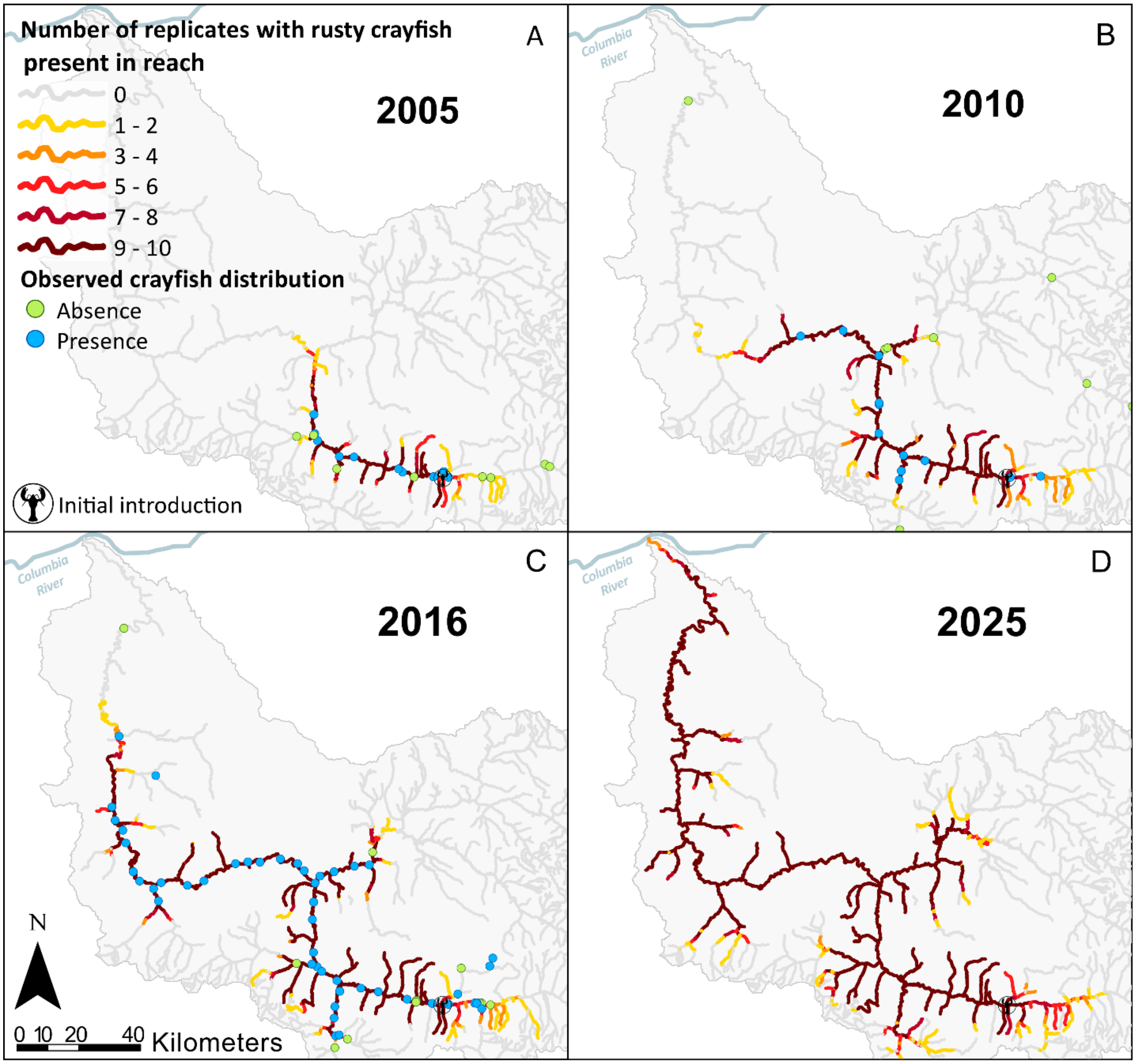

Figure 1.2. Model predictions and known occurrences of rusty crayfish in the John Day River basin. Invasion spread of rusty crayfish in the JDR basin in each river reach (A) 6 years, (B) 11 years, (C) 17 years, and (D) 26 years after their putative date of introduction with model uncertainty represented as the number of replicate model runs predicting crayfish presence in each river reach (colored lines).Three sets of survey data on rusty crayfish presence (blue points) and absence (green points) were used to train the model and assess the degree to which the predicted distribution matched the historical spread of rusty crayfish. Given the lack of absences at expanding edges of the invasion [e.g. downstream in (A) 2005 and (B) 2010], the model was calibrated so that crayfish spread exceeded the furthest recorded presence.

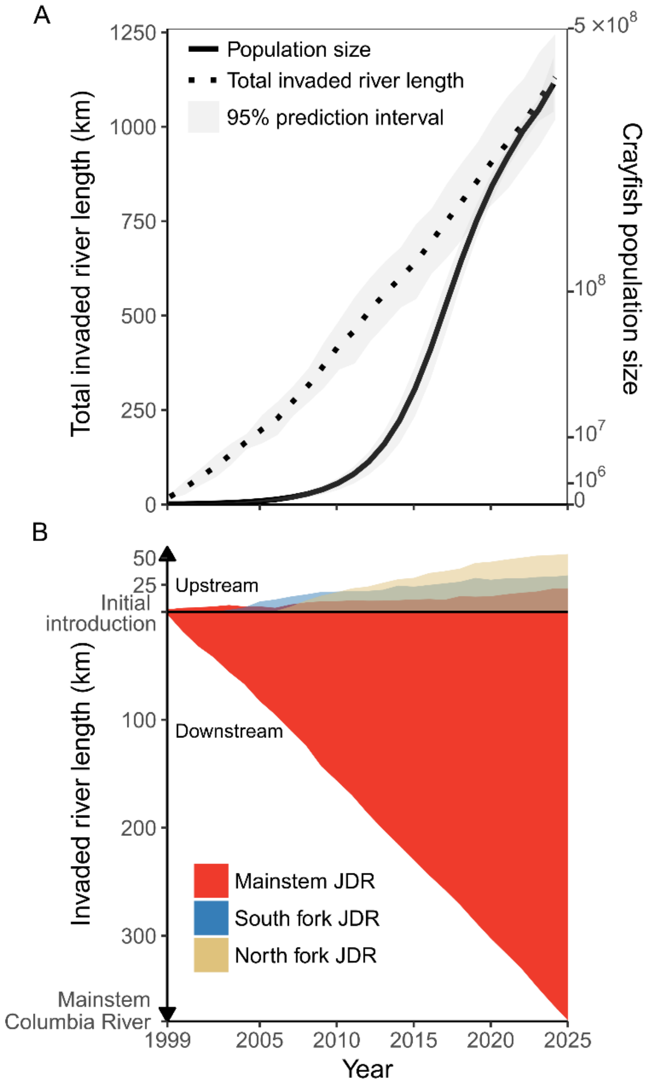

Figure 1.3. Modeled growth and spread of the crayfish population in the John Day River basin.

(A) Temporal trends in total invasion extent (left y-axis) and population size (right y-axis) of rusty crayfish in the John Day River (JDR) basin from their putative date of introduction (1999) to present-day (2016) to future (2025). Crayfish population size is square-root transformed. (B) Total invasion extent of rusty crayfish from the putative introduction location and date downstream (below the horizontal bar) and upstream (above the horizontal bar) in the mainstem, South Fork, and North Fork of the JDR. The start date of the shaded regions for the South Fork and North Fork JDR illustrate the initial modeled occurrence of rusty crayfish in these tributaries.

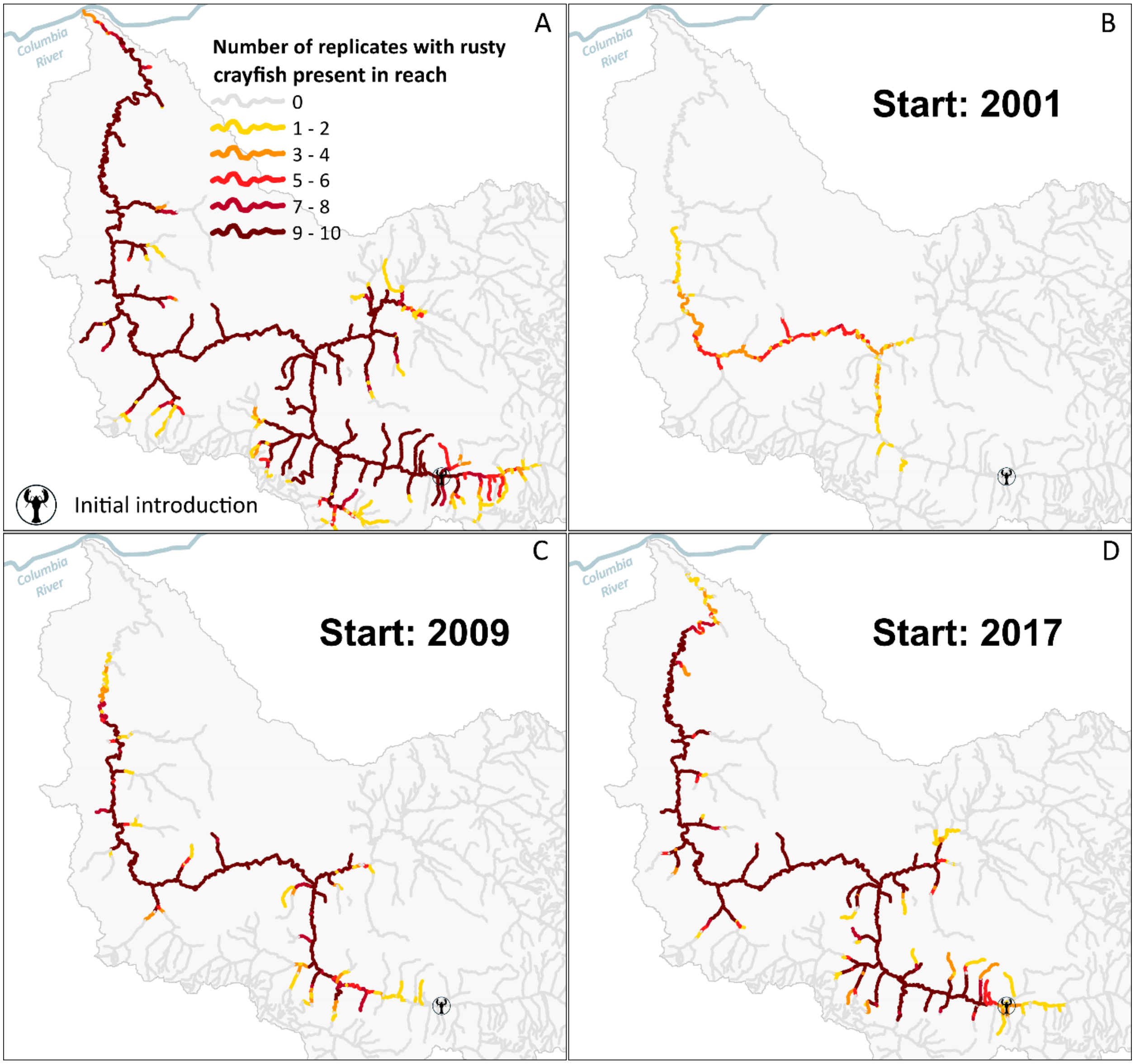

Figure 1.4. Modeled effects of control strategies on the distribution of rusty crayfish in 2025 in the John Day River basin. Comparison of the projected invasion extent of rusty crayfish in the JDR basin in 2025 (A) without any management action and under three scenarios of crayfish control, consisting of trapping 50% of the population in every reach yearly starting in (B) 2001, (C) 2009, and (D) 2017. Model uncertainty is represented as the number of replicate model runs predicting crayfish presence in each river reach (colored lines).

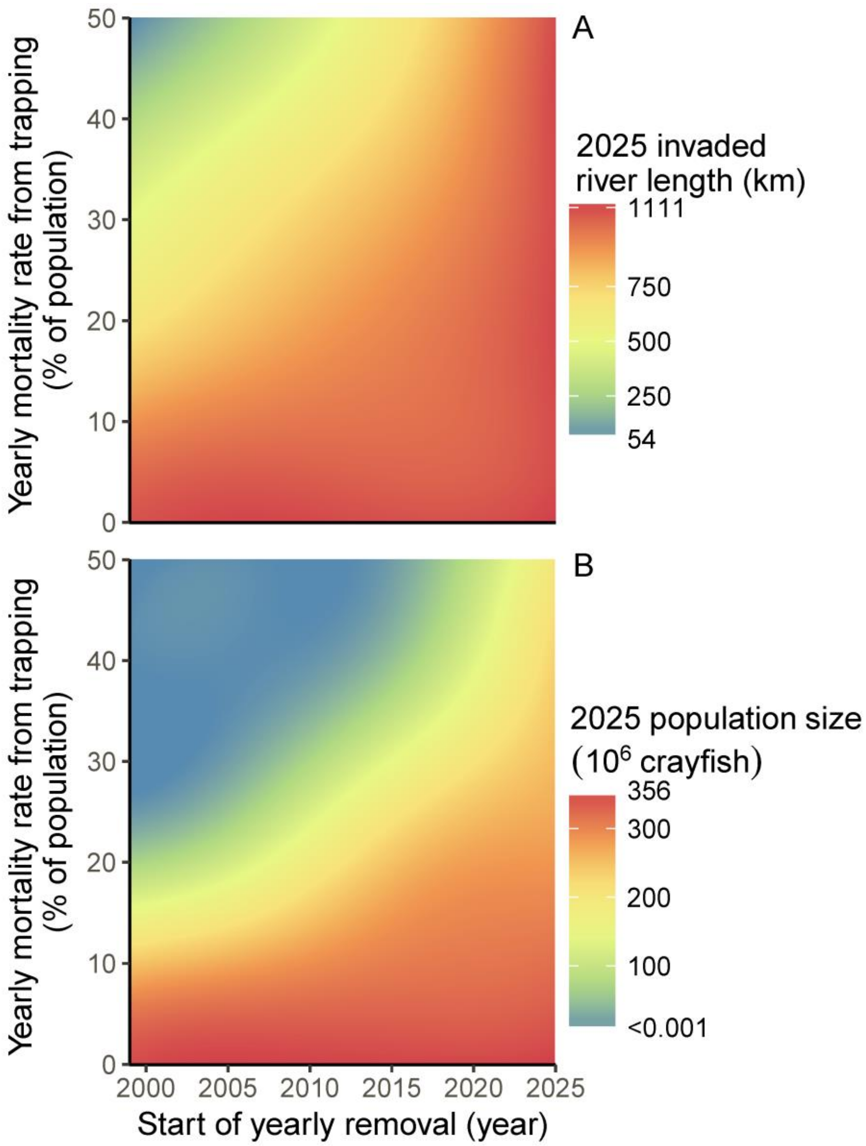

Figure 1.5. Effectiveness of different trapping removal efforts (y-axis) and years of initiating removal efforts (x-axis) on reducing (A) the length of river invaded by rusty crayfish and (B) the size of the rusty crayfish population in 2025. The heat maps were interpolated from mean values calculated over 10 replicates for each control scenario by universal kriging, based on Gaussian models of the empirical variograms.

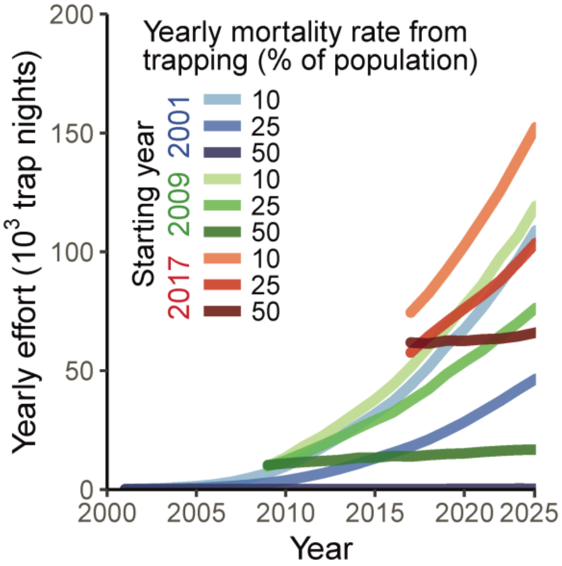

Figure 1.6. Projected yearly trapping effort required to achieve mortality rates from control strategies. Temporal trend (x-axis) in yearly trapping effort (averaged over 10 replicate model runs, y-axis) required to achieve increasing levels of population reduction (additional yearly mortality from trapping, lighter to darker colors) in each reach of the John Day River basin. Effort is measured in trap night representing one trap set for 12h overnight. Therefore, 1000 trap nights can be 1000 traps set over one night or 100 traps set over 10 nights. Starting earlier (e.g. two years after the putative year of introduction, blue hue) requires less cumulative effort in the long-term than starting 10 years (green hue) or 18 years (red hue) after the initial introduction. Similarly, investing more resources upfront (higher mortality rate, darker colors) decreases long-term costs due to more effective control of population levels.

1.11 Supplementary information

1.11.1 Appendix 1.1: water temperature and channel width model development

1.11.1.1 Channel width model

River width (m) for each reach was modeled based on downstream hydraulic geometry (Leopold & Maddock, 1953) with a log-log linear regression between mean annual discharge (m^3/s) from the NHDPlus v2 and satellite imagery-derived estimates of river width (m), similarly to Downing *et al.* (2012). Wetted width on ArcGIS™ basemap imagery was measured, and then averaged, for three locations (50 m downstream, 50 m upstream, and at the center) in each of 230 randomly selected reaches throughout the John Day River (JDR) basin. When limited visibility from vegetation precluded measurement at a given location, wetted width was measured at the nearest location with sufficient visibility within 250 m of the initially planned measurement location. For each reach included in the analysis, the average of these three measurements was used in the regression. The stream elevation at the upstream end of each stream segment (m) was used as a predictor variable in addition to mean annual discharge to account for the influence of variable landscape positions in the watershed.

The equation of the selected regression model ($R^2=0.89$, $P<0.001$) was:

$$\log_{10}(\text{wetted width m})=0.56+0.38\times\log_{10}(\text{mean annual discharge } m^3/s)-3.9\times10^{-6}\times\text{elevation (m)}$$

The surface area (m^2) of each reach was then computed as the product of its estimated width and the NHDPlus v2 length, and used in our model to compute crayfish density in each river reach.

1.11.1.2 Water temperature model

Average daily water temperatures from 1999 to 2016 were estimated for each reach following McNyset, Volk, and Jordan (2015). Publicly available daily summaries of hourly water temperature data collected between 1999 and 2010 by tribal, state, and federal organizations at nearly 700 sites throughout the JDR watershed and compiled as part of the Integrated Status and Effectiveness Monitoring Program (http://isemp.org/products/explore-data/historical-isemp/) were used as training and validating data for model development. Non-spatial multiple regression models relating average daily water temperature (°C) to a suite of predictors were built separately for spring and autumn of 1999 and 2000-2016, for a total of four models.

The main candidate predictor variables of stream water temperature were 1-km resolution remotely-sensed mean daily land surface temperature (LST in °C from the Moderate Resolution Imaging Spectroradiometer, MODIS, available at https://modis-land.gsfc.nasa.gov/temp.html) for the period 2000-2016, and daily air temperature recorded at seven weather stations within and around the JDR basin for 1999 (LST data did not exist prior to the year 2000). Other candidate variables included Calendar day (1-366), watershed area (km^2), mean elevation within watershed (m), stream elevation (m) and slope (%), forest cover within watershed (%), forest and woody wetland cover within 100 m of river reach (%, riparian cover), and housing density within watershed (housing units/km^2) from StreamCat (Hill, Weber, Leibowitz, Olsen, & Thornbrugh 2016) – available at https://www.epa.gov/national-aquatic-resource-surveys/streamcat. Final models were selected by iteratively adding or removing variables and comparing model performance based on adjusted-R^2, Root-Mean-Squared-Error (RMSE), and Akaike Information Criterion (AIC). Multicollinearity was assessed using the Variance Inflation Factor (VIF), together with observations of coefficient parameters (Table 1.S1).

For both periods, the final models included air/land surface temperature, calendar day, watershed area, and stream elevation as predictor variables. We used these trained models to dynamically predict daily water temperatures in each reach throughout the watershed from 1999 to 2016 and used mean predicted daily water temperatures from 2012-2016 (representing 5 out of the 10 hottest years over the study period) to simulate water temperatures from 2017 to 2025. Finally, daily temperatures were aggregated at the monthly time step to be incorporated in the model by computing the number of days in each month from 1999-2025 when water temperature in a given reach was > 6°C, the threshold under which rusty crayfish become mostly inactive (see Appendix 1.4).

Table 1.S1. Equations and performance indices of water temperature models of increasing complexity for 1999 and 2000-2016. Bolded equations highlight the four selected models for Spring and Fall of the two time periods. The maximum variance inflation factor (VIF) does not include that for polynomials (of air temperature and land surface temperature) as collinearity is systematically present among these predictor variables and is thus not necessarily representative of multicollinearity among other variables. AT: daily mean air temperature from the Blue Mountain Spring weather station (°C) (https://www.ncdc.noaa.gov/cdo web/datasets/GHCND/stations/ GHCND:USS0018E16S/detail), CD: Calendar Day, WA: Watershed area (m^2), elv: Stream elevation (m), slo: reach slope (%), HUDen: housing density (housing units per square kilometer within watershed), PctFstWs: watershed forest cover (%), LST: daily mean Land Surface Temperature from MODIS (°C), PctFstRp: riparian forest cover (%).

Year	Period	Model	R^2	RMSE	AIC	Max VIF
1999	Spring	AT	0.48	4.02	32722	-
		AT + AT^2	0.49	3.98	32633	-
		AT + AT^2	0.59	3.68	12976	-
		AT + AT^2 + CD	0.63	3.52	12767	-
		AT + CD + $\log_{10}$(WA)	0.77	2.78	11652	1.1
		4.86 + 2.62·10^{-1}·AT + 5.56·10^{-2}·CD + 4.81·10^{-1}·$\log_{10}$(WA) – 5.26·10^{-5}·elv	**0.82**	**2.43**	**10999**	**3.0**
		AT + CD + $\log_{10}$(WA) + elv + √slo	0.82	2.43	11000	7.2
		AT + CD + $\log_{10}$(WA) + elv + HUDen	0.82	2.43	11000	4.7
		AT + CD + $\log_{10}$(WA) + elv + PctFstWs	0.83	2.40	10954	5.5
	Fall	AT + AT^2	0.38	4.15	19555	-
		AT + AT^2 + CD	0.45	3.91	19150	2.8
		AT + CD + $\log_{10}$(WA)	0.73	2.74	16711	2.7
		33.5 + 2.43·10^{-1}·AT – 8.50·10^{-2}·CD + 1.14·$\log_{10}$(WA) – 4.20·10^{-5}·elv	**0.78**	**2.50**	**16076**	**3.3**
		AT + CD + $\log_{10}$(WA) + elv + √slo	0.78	2.50	16063	8.7
		AT + CD + $\log_{10}$(WA) + elv + PctFstWs	0.78	2.48	16014	6.4
2000-2016	Spring	LST + CD	0.71	2.94	209033	2.2
		LST + LST^2 + CD	0.71	2.90	208037	3.1
		LST + LST^2 + CD + $\log_{10}$(WA)	0.78	2.58	197372	3.4
		LST + LST^2 + CD + $\log_{10}$(WA) + elv	0.78	2.52	196159	4.4
		LST + LST^2 + CD + $\log_{10}$(WA) + elv + √slo	0.78	2.54	196818	4.4
		LST^2 + CD + $\log_{10}$(WA) + elev + √slo	0.79	2.51	195871	3.7
		LST^2 + CD + $\log_{10}$(WA) + elev + √slo + HUDen	0.79	2.51	195845	3.7
		LST^2 + CD + $\log_{10}$(WA) + elev + √slo + PctFstRp	0.79	2.49	195271	3.7
		LST^2 + CD + $\log_{10}$(WA) + elev + √slo + PctFstWs	0.79	2.49	195028	3.7
		-2.28 + 2.20·10^{-3}·LST^2 + 7.74·10^{-2}·CD + 1.44·$\log_{10}$(WA) - 1.92·10^{-5}·elv	**0.79**	**2.51**	**196168**	**2.6**
	Fall	LST + CD	0.65	2.87	259292	2.3
		LST + LST^2 + CD	0.66	2.83	258065	2.6
		LST + LST^2 + CD + $\log_{10}$(WA)	0.77	2.36	238843	2.8
		LST + LST^2 + CD + $\log_{10}$(WA) + elv	0.78	2.30	235997	3.3
		LST + LST^2 + CD + $\log_{10}$(WA) + elv + √slo	0.78	2.30	235997	3.3
		LST + LST^2 + CD + $\log_{10}$(WA) + elv + HUDen	0.78	2.28	235086	3.3
		LST + LST^2 + CD + $\log_{10}$(WA) + elv + PctFstWs	0.78	2.26	234373	3.3
		29.2 + 1.43·10^{-1}·LST - 8.42·10^{-2}·CD + 1.53·$\log_{10}$(WA) – 2.35·10^{-5}·elv	**0.78**	**2.30**	**236273**	**3.4**

1.11.2 Appendix 1.2: conceptual diagram of the individual-based model

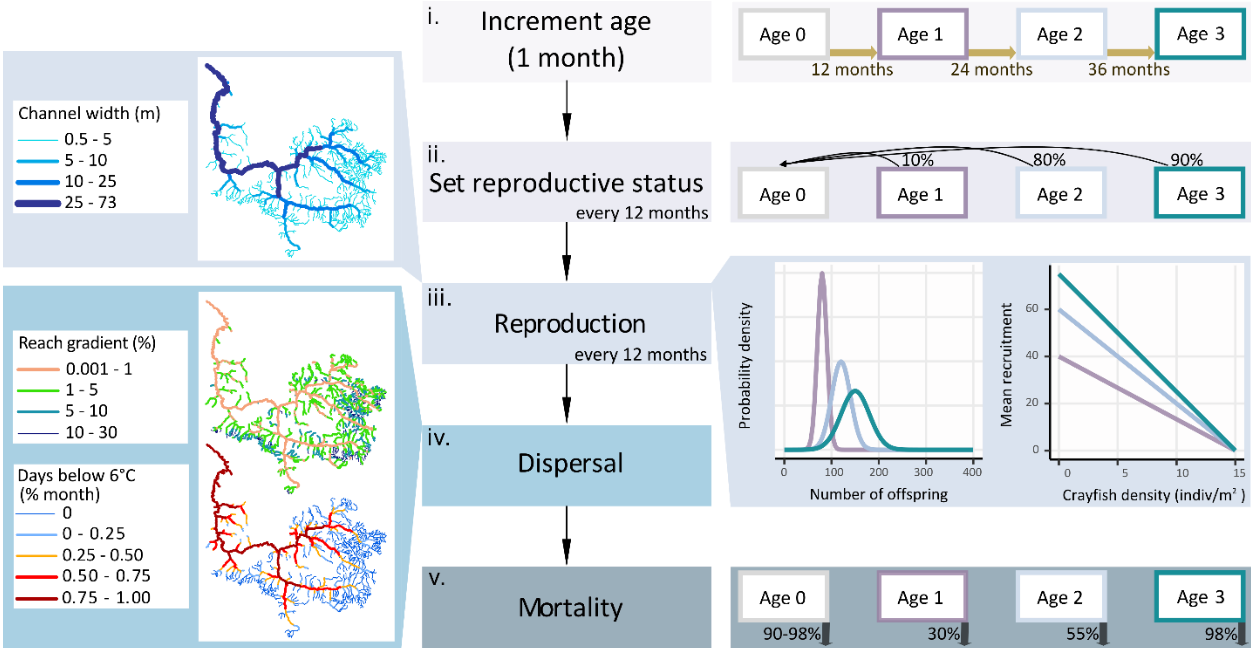

Figure 1.S1. Conceptual diagram of the basic architecture of the spatially explicit individual-based model for rusty crayfish in the John Day River basin.

All crayfish in the model undergo a four-age class life cycle that consists of a portion of the population moving and dying every month and mating yearly (central column), taking place in a spatially explicit stream network (left-hand column). All crayfish age every month and transition to the next age class yearly in June (i. right). Every year in July, a percentage of individuals in every age class (ii. right) produce a number of offspring drawn from a normal distribution whose mean and standard deviation depend on the parent's age and the density of crayfish in their reach (iii. right). The density of crayfish in a given reach is computed as the number of individuals in the reach divided by the product of the reach length and modeled width (ii. left, Appendix 1.1). Every month, each crayfish then disperses across the network based on a dispersal kernel (Appendix 1.6), as well as the gradient and the water temperature of the reach it is moving through (iv. left). Finally, every month, an age class-dependent percentage of the population dies (v. right). The numbers in the diagram (right-hand column) are for all crayfish for the whole year while the model was parameterized as female-only, monthly, and included super-individuals. Parameters in the diagram were derived from a systematic review of the literature as well as inverse modeling and reflect those used in the final model of rusty crayfish spread (Appendix 1.4 and 1.5).

1.11.3 Appendix 1.3: bibliography from literature review on rusty crayfish

We conducted a systematic literature review for rusty crayfish, *Orconectes rusticus*, by selecting all articles published between 1900 and 2016 that mentioned orconectid crayfish or rusty crayfish in their abstract from searching (rusty crayfish) OR (*Orconectes rusticus*) in Web of Science and JSTOR. We read all selected works and further searched for relevant work using a snowball approach of analyzing the bibliography of the papers. This process yielded a total of 210 relevant documents including peer-reviewed articles, books, and graduate theses on the biology, behavior, and ecology of rusty crayfish, listed below.

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1.11.4 Appendix 1.4: literature review used in determining model structure and parameters

The biology and ecology of rusty crayfish have been extensively studied in the literature. A systematic literature review of the literature (Appendix 1.3) yielded a substantial body of research from which the structure and parameters of the HexSim model were derived. The following is a summary of this review and the rationale behind the HexSim model structure and parameter values.

1.11.4.1 Female-only model

The HexSim model developed in this study relied on a female-only structure. A female-only model implies that fecundity values are halved and that the model overall population abundance corresponds to female abundance (Hein, Roth, Ives, & Vander Zanden 2006). Moreover, pair interaction for mating and hence Allee effects [e.g. population growth failure when densities are too low to allow for mate pairing (Keitt, Lewis, & Holt 2001)] are not taken in account. Developing female-only models is a common practice that allows for reduced computation time and was previously adopted for both a crayfish population model (Hein *et al.*, 2006) and for other species in previous examples of HexSim models (Huber, Greco, Schumaker, & Hobbs 2014; Marcot, Singleton, & Schumaker 2015). Evidence of sex ratios departing from 1:1 are found at crayfish invasion vanguards both with females being over-represented (Perry, Feder, Dwyer, & Lodge 2001; Pârvulescu, Pîrvu, Moroşan, & Zaharia 2015) and males being found in greater proportion (Rebrina, Skejo, Lucić, & Hudina 2015). However, evidence suggests that the ratio of adult males to females is 1:1 in established populations of *Orconectes rusticus* (Lorman, 1980; Hamr, 2010), based on the sex ratio in juveniles and that of adults during inactive periods (Prins, 1968; Hamr, 1997).

1.11.4.2 Model initialization

Observed crayfish densities in the few years following the introduction, a regional survey of potential crayfish distributors, and testimonies from school employees, suggest that the rusty crayfish propagules (first introduced individuals) might have originated from a release by a nearby school in the town of John Day, Oregon (Larson & Olden, 2008; Olden, Adams, & Larson 2009). However, other modes of introduction such as bait bucket discharge from recreational anglers, a common vector of crayfish introduction (DiStefano, Litvan, & Horner 2009), cannot be discounted given the proximity of a popular smallmouth bass fishery and nearby Magone lake invaded by rusty crayfish for an unknown period of time (Miller & Sytsma, 2014). Based on this evidence, we initialized the model with 5-30 propagules, a reasonable quantity for either a biology class or a bait bucket. The final model was initialized with 10 female propagules.

1.11.4.3 Age classes

Most crayfish of the genus *Orconectes* live 3-4 years (Holdich & Crandall, 2002). In a Kentucky stream, Prins (1968) states that the majority of rusty crayfish die in 2.5 years though some slower growing individuals live over three years. In Ontario, Corey (1988) observed the predominance of year I and II crayfish with a very low survival rate into the fourth year (2-5%) in a lake population and found no such old crayfish in a nearby river population. Hamr (1997) also found three to four year classes, with the majority being three year olds based on carapace length frequency histograms. Finally, in their seminal work on the biology of rusty crayfish Lorman (1980) established that less than 3% of those crayfish that reach maturity survive until the end of their fourth year (48 months) in Sugarbush Lake, Wisconsin. Following these observations and their own, Hein *et al.* (2006) also developed a four-stage Leslie matrix model of rusty crayfish.

Empirical data from the population under study in the JDR suggest that there was only a maximum of three age classes in the newly established rusty crayfish population at the time of sampling (Sorenson, 2012). Sorenson (2012) hypothesized that the JDR might not be an ideal habitat for rusty crayfish given generally low temperatures in this system for most of the year. However, several crayfish specimens over 40 mm in carapace length were found in July of 2010 and August 2016. In addition, kick-netting is known to be slightly biased towards medium and small crayfish with a small capture rate of larger crayfish (Price & Welch, 2009).

In this study, the crayfish life cycle and population was divided in 4 life age classes. Rather than defining stages according to sizes at age, we defined classes based on age (number of months since the onset of stage III juvenile -- independence from mother) whereby crayfish transition from age 0 to age 3 every 12 months in Spring.

1.11.4.4 Reproductive status

A “reproductive status” trait was included in the model to assign whether a given crayfish female was going to successfully reproduce. In the model event sequence, the setting of an individual’s reproductive status was performed through a transition event once a year. This event sets the reproductive status of each individual to either “reproductive” or “non-reproductive” based on a probability that varies based on the crayfish age. For instance, the probability of age 0 crayfish to become reproductive is null while age 1 crayfish have a 10% of becoming reproductively active (see below).

- *Age 0:* Age 0 crayfish are not present during recruitment in June (every 12 months starting from month 0) as they only become independent from their mother in July

- *Age 1:* size and age at onset of sexual maturity vary within and between rusty crayfish populations. Spawning in rusty crayfish takes place during September and October as well as in late winter/early spring [February-March (Prins, 1968) and as late as April in cold waters (Hamr, 1997)] with variation between populations and years. To spawn either in autumn or in spring, young-of-the-year (YOY) crayfish need to have reached sexual maturity (molted into form I condition for males and glair development for females) by the end of their first summer, as low temperatures interrupt molting and therefore growth from late autumn to early spring (Prins, 1968; Hamr, 1997). It is sometimes observed that the fastest growing YOY can reach sexual maturity by the end of their first summer or the first molt of the spring season and spawn in Autumn and/or Spring (Langlois, 1935; Prins, 1968; Lorman, 1980; Hamr, 1997). However, the majority remain sexually immature until the following summer. In the JDR, the smallest ovigerous female observed on May 30th 2010 by Sorenson (2012) was 21.6 mm, no ovigerous female was observed on April 24th 2011 and one ovigerous female only (CL = 23.7 mm) was observed on May 31st. Their analysis shows that the mean YOY carapace length at the end of the growing season (end of October) is about 14 mm, and that a very small percentage of YOY grow beyond 18-20 mm by that time. Evidence from the literature and the JDR therefore suggest that only a very small percentage of YOY reach sexual maturity by the end of their growing season and proceed to become ovigerous the following spring. Therefore, only 10% of age 1 (10 months +) crayfish participate in recruitment in our model. In subsequent developments of the model, growth based on degree days could be computed based on Lorman’s (1980) equations to determine at what age crayfish reach maturity in streams of the JDR based on temperature.

- *Age 2 and 3:* Estimating the percentage of ovigerous females and their fecundity in terms of juvenile recruitment is a challenge due to their seclusion in burrows and shelters during the period starting with egg extrusion until juveniles become independent (Corey, 1988). Langlois (1935) found over 80% of adult females in berry in Ohio ponds. Prins (1968) notes that at any one time, up to 34% of adult females collected were in berry in the Kentucky stream under study. Of 1000 mature females collected over three brooding seasons, 82.1% carried eggs or young (95%CI: 79.6-84.3) in Upper Sugarbush Lake, Wisconsin, with percentage with eggs or young peaking at 96% (n = 75) on May 27th 1976, 92.9% (n = 212) on May 21st 1977, and 100% (n = 12) and 93.2% (n = 118) on June 5th and 8-11th 1978, respectively (Lorman, 1980). Lorman (1980) also pointed out that “a higher percentage probably reproduced successfully, since that figure includes some individuals that had not yet laid eggs or had already released their young”. Of the mature females in Corey (1988)’s samples, 48.0 to 83.9% and 66.7 to 90.5% were ovigerous in the river and lake populations, respectively, after oviposition in early April. Hamr (1997) observed that 95% of mature females in samples shower glair development by September and 88% of mature females in their samples had spawned by mid-April. In the Upper Susquehanna River watershed, Kuhlmann (2008) drew a logistic regression determining the probability for a female to be gravid based on carapace length and found that the length at which a female was 50% likely to produce offspring was about 23mm. From their graphs, we can approximate a 60% chance at 25 mm, and 85-90% chance at 30 mm. Given the paucity of data on ovigerous females in the JDR, little can be inferred on the percentage of ovigerous females. However, even with the small sample size collected by Sorenson (2012), it should be noted that 10 out of 12 females above 20 mm, and 10 out of 13 females above 18 mm were ovigerous on May 30th 2010, corresponding to 77% and 83% of the potentially mature females in the sample, respectively. Out of only 7 females collected on May 31th 2011, the only female of sufficient size to be mature was gravid (Sorenson, 2012). For our model, we therefore parameterized that 80% and 90% of crayfish would successfully spawn at Age 2 and 3, respectively.

1.11.4.5 Fecundity and life-cycle

Crayfish fecundity is evaluated in various ways in the literature: reproductive potential describes the number of ova in the ovaries, realized production alludes to abdominal egg counts, and actual production is determined through counts of stage III larvae/juvenile; reproductive efficiency is the percent survival from ova to stage III larvae (Corey, 1990). See Table 1.S2 and 1.S3, and Figure 1.S2 for a summary of the literature on clutch size and reproduction timing.

Usually, abdominal/pleopodal eggs are fewer than ovarian eggs as some eggs are not fertilized and losses occur during development. Discrepancies between pleopodal and ovarian egg counts are generally most pronounced in river populations and are attributed to the "rigors of the stream environment" – higher water velocity and molar action of bed sediment (Prins, 1968). This discrepancy, according to Prins (1968) increases with crayfish size to reach 30% of the ovarian count for the largest crayfish, hypothesizing that large clutches are more vulnerable. However, Momot and Gowing (1977b) found disparities between ovarian and abdominal egg counts as high as 50% in *O. virilis* and Mason (1975) also found that about 40% of ovarian egg production was lost before attachment. Regressions of number of pleopodal eggs within a week after oviposition and number of attached juveniles in Upper Sugarbush Lake suggest low rates of loss [less than 10% for 45 mm female crayfish; (Lorman, 1980)].

While Corey (1987) observed no apparent differences in fecundity across the range of rusty crayfish. We found large variations among systems and studies in our literature review. Several explanations can account for the widely varying fecundity relationships found in previous works. First, differences in habitat quality could lead to differences in crayfish fitness such that females could allocate more energy to reproduction. Habitat quality indicators potentially influencing reproductive effort include temperature, food quality, predator pressure, shelter availability, or crayfish density. Second, different populations of crayfish might have intrinsically different fecundity as introduction often leads to pioneer effects and secondary spread can lead to selection for some traits at the edge of the invasion. Third, while no clear pattern sets estimates for rivers apart from that for lakes, different stress levels among rivers and between lotic and lentic systems could lead to different rates of pleopodal egg loss. Finally, abdominal egg counts for sampling performed longer after oviposition will be lower than those performed immediately after oviposition due to egg losses [e.g. Lorman (1980) explicitly counted abdominal egg counts within one week after oviposition]. In the only study comparing a river and a lake population, Corey (1988) found that the lake population had a higher fecundity than the river population in Ontario, and that loss of eggs at oviposition was positively correlated to current velocities in rivers (unpublished data).

Another factor that influences variability in egg production among females is egg size. The standardized residuals of the regression of number of eggs based on female carapace length were negatively correlated with individual egg weight (Lorman, 1980), such that for a given carapace length, females that produce larger eggs produce few eggs.

In the JDR, Sorenson (2012) only found eleven ovigerous females over two seasons of sampling. The mean and maximum number of pleopodal eggs on these females, 96 and 140 respectively, is far inferior to that found in previous studies (with mean number of eggs around 150, and maximum number of eggs systematically over 250 and as much as 574). However, it is important to note that Sorenson (2012) collected relatively small ovigerous females with carapace lengths only spanning 21.6 to 25.7 mm. By contrast, most studies rely on ovigerous females with over 20 mm differences in carapace length. Removing two outliers, we regressed the number of pleopodal eggs against carapace length for the remaining nine ovigerous females sampled in the JDR by Sorenson (2012) and found a similar relationship as (Corey, 1987; Corey, 1988) for pleopodal eggs and Prins (1968) for ovarian eggs.

In our model of rusty crayfish in the JDR, fecundity values correspond to actual production for females -- the number of fertilized gametes that hatch and become independent stage III female juveniles. Therefore, to base our estimate on pleopodal eggs, we need to account for further losses and decay of eggs through incubation.

In an age-structured population model, based on the regression of the number of attached juveniles against female length from Lorman (1980) and female crayfish size distribution in Sparkling Lake, Hein *et al.* (2006) assigned mean fecundity (as the number of female stage III juveniles) of 44.5, 62.5, and 81.5 for age 1, 2, and 3 respectively. Given that their model is female-only, these correspond to mean total fecundity of 89, 125, and 163. Arcella, Perry, Lodge, and Feder (2014) found that at the time of hatching, the mean number of offspring (n = 75) for female rusty crayfish in Trout Lake, Wisconsin was 68 (standard error = 10), corresponding to a female-only actual production of only 34.

Based on this information, initial fecundity values for our model were determined as follow:

- Age 1 mature female crayfish (~17-20 mm) generally extrude 50-100 pleopodal eggs, undergo 10% egg and juvenile loss, which leads to 45-90 stage III juveniles. Therefore, we set age 1 mean fecundity at 40 female recruits with a standard deviation of 5 recruits.
- Age 2 mature female crayfish (~20-27 mm) generally extrude 100-150 pleopodal eggs, undergo 10% egg and juvenile loss, which leads to 90-135 stage III juveniles. Therefore, we set age 2 mean fecundity at 60 female recruits with a standard deviation of 10 recruits.
- Age 3 mature female crayfish (~25-45 mm) generally extrude 150-250 pleopodal eggs, undergo 20% egg and juvenile loss, which leads to 110-210 stage III juveniles. Therefore, we set age 3 mean fecundity at 75 female recruits with a standard deviation of 20 recruits.

Table 1.S2. Summary of quantitative estimates for rusty crayfish (*Orconectes rusticus*) fecundity in the literature. Syst. refers to whether a study was in a lotic or lentic environment, ovar. to ovarian eggs, pleop. to pleopodal/abdominal eggs, CL to carapace length, n number of samples, R^2 is the proportion of variance explained by the equation. To convert total length to carapace length for Langlois (1935) data, we used relationships previously derived in the literature by Corey (1987): CL = 0.467TL – 2.186 (r = 0.9946, N = 50).

Syst.	**Type**	**Mean #eggs**	**Mean CL**	**Range #eggs**	**Range CL**	**Equation (eggs-CL mm)**	**n**	**R^2**	**Reference**
Lotic	ovar.	-	-	54-357	18-37	12.50 CL - 160.49	43	0.92	Prins (1968)
Lentic	pleop.	150.6	21.7	47 - 332	13.9 - 33.7	13.1 CL - 132.9	41	0.88	Corey (1988)
Lentic	pleop.	-	-	-	-	3.772 CL - 27.2	221	0.30	Lorman (1980)
Lentic	pleop.	-	-	-	-	exp(0.0329 CL + 1.0636)		0.58	Hein *et al.* (2006)
Lentic	pleop.	-	-	-	-	exp(0.0357 CL + 1.0672)		0.75	Hein *et al.* (2006)
Lentic	pleop.	276	34.9	80-574	19-45	17.42 CL - 278.08	6	0.86	Langlois (1935)
Lotic	pleop.	-	-	42-231	17-38	8.31 CL - 91.49	49	0.86	Prins (1968)
Lotic	pleop.	161.8	26	75-351	14-36	12.51 CL - 135.36	52	0.74	Corey (1987)
Lotic	pleop.	143.2	23.5	35-351	17.2 - 32.7	11.5 CL - 126.2	44	0.70	Corey (1988)
Lotic (JDR)	pleop.	96	23.3	25-140	21.6 – 25.7	13.316 CL – 214.4	9	0.83	Sorenson (2012)
Lotic	pleop.	165	29.05	76-256	22.8 - 33.8	4.1963 CL + 43.222	8	0.09	Loughman, Simon, and Welsh (2009)
Lentic	YOY	-	-	-	-	3.425 CL - 20.5	134	0.18	Lorman (1980)
Lentic	YOY	68	28.47		18 - 48	-	75	-	Arcella *et al.* (2014)

Table 1.S3. Summary of information on rusty crayfish *Orconectes rusticus* reproductive timing from literature.

Oviposition	Time to hatching	Hatch	Stage I and II	Stage III (recruitment)	References
March to June (mostly April and May)	4-6 weeks (temp.dependent)	May	3 weeks	Early June	Prins (1968)
By late April (all early May)	-	mid-late May	mid-June (start May 25th)	-	Hamr (1997)
Early April	6-8 weeks	-	-	Early to late June (depends lake vs river)	(Corey 1988)
March-April	about 3-4 weeks	1-2 weeks	June-early July	-	Butler and Stein (1985)
April - May (depends on temp.)	-	Late April		May 1st (not possible given hatching date)	Langlois (1935)
Mid April - early May (only time sampled because of ice-thaw)	May-June (mean hatching dates May 22-June 7; depends on temp., 95% hatched within 16 days)	-	-		Lorman (1980)
Ovigerous females in May	-	-	-	Still some juveniles attached in July	Sorenson (2012)
-	20 days (20-22C)	-	-	-	Busch (1940)

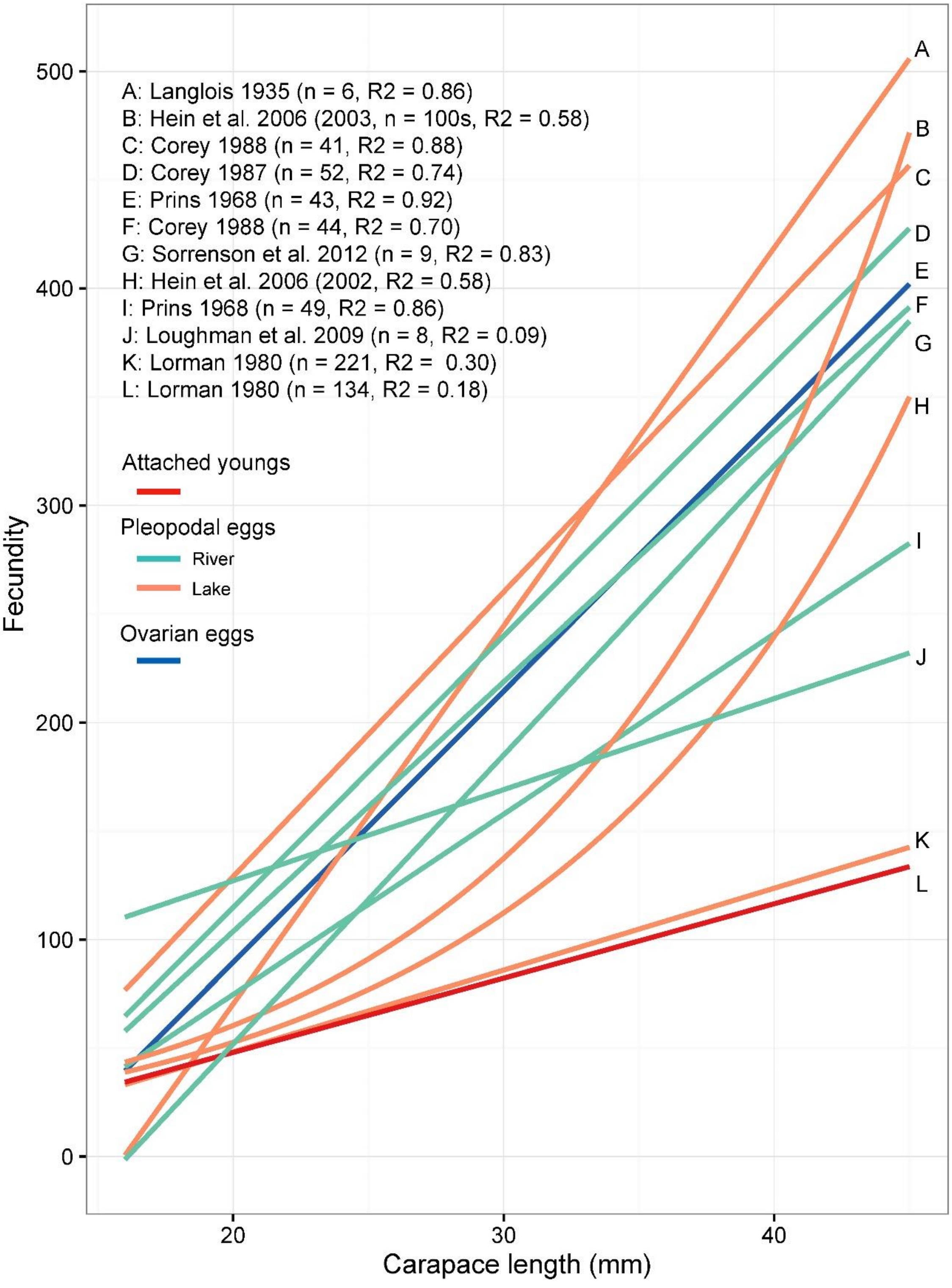

Figure 1.S2. Compilation of carapace length (x-axis)-fecundity (y-axis) relationship for rusty crayfish *Orconectes rusticus.*

1.11.4.6 Density dependence

Our model includes density dependence as a linear relationship between the density of crayfish in a given reach and the number of young of the year recruits. The value at which recruitment was null was determined based on values reported in 30 studies and our own observations, though values widely varies among and within systems in the literature. Even after removal of large portions of the population in Sparkling Lake, Wisconsin, Hein *et al.* (2006) observed no compensatory changes in female fecundity, which suggests that density dependence is not strong. Regressing juvenile rusty crayfish density against adult female density using data from multiple lakes, Hansen, Ives, Vander Zanden, and Carpenter (2013b) also found that this relationship was linear and that the best-fit parameter estimated for both nonlinear stock-recruitment models included zero, pointing to a potential absence of density dependence. Nevertheless, Hansen, Ives, Vander Zanden, and Carpenter (2013a) included density dependence in their population model and Momot and Gowing (1977a; 1977b) found that the number of attached eggs decreased with higher densities of *O. virilis* in two lakes of Michigan.

1.11.4.7 Survival

Most rusty crayfish live 2 years with some living over 3 years. Overall, survivorship is extremely low for YOY (age 0), higher for age 1 individuals, low for age 2 and even lower for age 3 individuals (Hein *et al.*, 2006). Prins (1968) found that most rusty crayfish over 25 mm died during the third spring and summer and Corey (1988) found that a very small percentage of crayfish (2 to 5%) survived a fourth year.

Mortality can result from predation, disease, physiological aging, molar action from bed sediment, or physiological stress from high temperatures. A large proportion of deaths occur during molting periods as molting results in physiological stress and vulnerability to predation and crushing during the solidification of a new exoskeleton. Therefore, periods of high flow (e.g. spring) or temperature can both result in high rates of mortality. Mortality also varies between systems based on shelter availability, with cobble habitat providing the most shelter from predation and allowing for greater densities and survival (Kershner & Lodge, 1995). Mortality is likely to be density dependent (Momot, 1984). Momot and Gowing (1977a; 1977b) found decreased survival of young-of-year *O. virilis* crayfish with increasing density and highly variable mortality rates among years and lakes in Michigan for *O. virilis.* Hansen *et al.* (2013a) found that lake water level was a potentially strong driver of rusty crayfish recruitment in Sparkling and Trout Lake, Wisconsin, hypothesizing that this might be attributable to a reduction in cobble habitats and a resulting increase in predation on juveniles from lack of shelter. Survival of rusty crayfish YOY was also greatly variable depending whether they came from their invaded or native range (Arcella *et al.*, 2014).

Mortality rates in laboratory experiments are highly dependent on densities, food, and temperature. For age 2 (CL = 26-30 mm) rusty crayfish, mean mortality during a 6 week experiment with no predator was ~0.2 and 0.4 with predator (non-consumptive effect) when held with 3 other crayfish at a density of 25 crayfish/m^2 (Hill & Lodge, 1999).

For *O. virilis,* Momot (1984) reports survival rates of 0.05 for YOY, 0.17-0.29 for males 1-2 years, 0.42-0.63 for males 2-3 years, and 0.48 for females 2-3 years as well as, for *O. propinquus* 0.05, 0.07, 0.20 for 0-1, 1-2, and 2-3 years (from Vannote and Ball 1963).

Hein *et al.* (2006) in their age-structure model used two different methods and Lorman (1980) data to compute survivorship. The first was based on dividing age-specific population estimates in September by those in May, based on the assumption that most mortality occurred during summer owing to molting and increased predation by fish, citing Lorman (1980). Juvenile survivorship was estimated by comparing population estimates from June to September. The second method computed survivorship from a single month age distribution assuming that the population was at stationary. These two methods resulted in two sets of survivorship rates for each age class. 1.6-3.2% for age 0, 65.2-70.8% for age 1, 36.3-60.7% for age 2, 9.1-12.8% for age 3. However, they caution of wide variations in net population growth during sensitivity analysis indicating that these estimates should be refined. These figures contrast with those detailed in (Lorman, 1980) according to which 3% of the estimated number of YOY survive to become mature at the end of the second growing season (age 0 and 1 survivorship), 20% of these survive to reproduce a second year (age 2 survivorship), and less than 3% survived a fourth year (age 3 survivorship).

Considering the variability in estimates of mortality, the exact value of the survival parameters in the model were refined based on iterative values while keeping fecundity and reproductive status constant.

1.11.4.8 Recorded speeds

While a relatively large amount of information is available on the habitat requirements of crayfish, less is known about their propensity to move and rate of movement. Even fewer estimates of rusty crayfish dispersal rates exist that are specific to river systems, as most studies focus on lentic systems. However, as Dresser and Swanson (2013) point out, rusty crayfish are more likely to naturally invade new territories through stream systems, such that more studies are needed to better understand the drivers of crayfish spread in lotic environments.

Crayfish movement is studied at various temporal and spatial scales. One approach relies on studying rates of secondary spread of non-native species over the course of months to years temporally and hundreds of meters to several kilometers spatially. This is done either through mark-recapture and radiotelemetry or through survey of distributional changes. Another set of approaches investigates movement speed either in laboratory or field settings over the course of hours to days through direct observation or days to weeks through radiotelemetry and mark recapture. The main difference between these two approaches is that the latter does not necessarily pinpoint population-level spread and might only record routine movement associated with foraging, mating, and homing behaviors. Indeed, radiotracking of crayfish movement such as in Bubb, Thom, and Lucas' (2004) study generally shows signal crayfish (*Pacifastacus* leniusculus) remaining in a shelter for a few days to a few weeks, performing small movements to collect food, and intermittently moving to a new location to find another refuge. Therefore, it is difficult to state whether rates of movement reported in the latter type of studies can be extended to population-level spread measured in the former type of research.

Momot (1996) calculated speed of secondary spread of rusty crayfish in Ontario ranging from 0.9 to 3.7 km accounting for variations between lentic and lotic systems, upstream and downstream, among years, and within each category. In their study, upstream movements range 0.45-1.5 km/year while downstream movements go from 0.05 km/year to 3.7 km/year when the smallest spread rates are associated with series of beaver dams, and from 0.9 to 3.7 km/year in the absence of obstacles. From trap catches in Trout Lake, Perry *et al.* (2001) estimated that the leading edge of rusty crayfish morphotypes had advanced an average of 0. 7 ± 0.1 (SE) km per year along the eastern shoreline and Wilson *et al.* (2004) later found that the rusty crayfish had dispersed around the entire shoreline of Trout Lake over 16 years at an average rate of 0.68 km/year (± 0.14 SE). At a smaller scale, through a 3-day mark-recapture study, Perry *et al.* (2001) observed that rusty crayfish mean dispersal distance varied depending on travelled direction with crayfish recaptured south along the shoreline from their release point moved further, 103.7 ± 21.2 (SE) m than those found north (37 .8 ± 5.7 m), with 22 out of 50 crayfish that did not move.

Many mark-recapture studies are unfortunately limited by their span such that they cannot capture the full movement kernel. In other words, most long-distance movements (the tail of the movement kernel) cannot be recorded as individuals move beyond the area of recapture that is furthest from the initial marking location. This is the case for Perry *et al.* (2001) but also for Byron and Wilson (2001)'s study. In the same lake, at 20°C, Byron and Wilson (2001) found through mark-recapture trapping that mean distances traveled between release and recapture did not differ significantly between the 48-h recapture (mean ±SE: 58 ± 8.87 m, max: 220 m) and the 96-h recapture (54 ± 7.73 m), that mean distance traveled was similar for male, female, or both genders combined for either 48 h or 96 h after release, and that size did not affect the distance crayfish moved. However, they also concluded that their sampling design underestimated dispersal as it was too small to measure adequately the distance traveled in 96 h. They hypothesized that crayfish could be divided in two groups a regarding movements, one that stayed and one that moved (at least over the course of a few days) in agreement with the idea that crayfish stay in a shelter for a few days to weeks before moving to another site sometimes far away. Upscaling their lowest hourly movement rate (0.56 m/h) to yearly rate if crayfish dispersed every hour in June, July, and August [months of highest crayfish activity in Wisconsin (Lorman, 1980)], they determine that the potential invasion rate of 1.2 km/year, far more than those found that by Perry *et al.* (2001) and later Wilson *et al.* (2004). In a similar exercise, Wilson *et al.* (2004) calculated that if rusty crayfish moved at an average of 29 m/day over these three months, it could cover 2.7 km/year and might have encircled Trout Lake in 3 years only. In a study of the 40 year range expansion of rusty crayfish in Lake of Woods, Ontario, Jansen, Geard, Mosindy, Olson, and Turner (2009) estimate that for six time periods for which values can be reasonably calculated, rusty crayfish expanded its distribution within the lake by an average of 2.1 km per year (range 0.8-4.0 km), far more than previously observed in Trout Lake, and more similar to the estimated maximum potential invasion rate of 2.7 km/year calculated by Wilson *et al.* (2004). Based on figures from repeated surveys of *O. rusticus* extent in a NY watershed, Kuhlmann and Hazelton (2007) show very high interannual variability in spread rates upstream ranging from 0.5 km to 8 km/year. Sometimes, the invasion front of rusty crayfish have even been found to contract from previous advances. This is not only the case in Kuhlmann and Hazelton (2007) study (and might be due to a false absence) but was also observed in 2004 by Bobeldyk and Lamberti (2008) who found neither rusty crayfish nor native crayfish species 3 km downstream of Bond Falls (Lake outlet) in the Upper Peninsula of Michigan, even though Charlebois and Lamberti (1996) found low densities of both species at the same distance in 1992. This pattern was confirmed by decreasing catch per unit effort going downstream, characteristic of an invasion edge.

The maximum recorded rate of invasive spread of rusty crayfish to date is therefore 8 km/year (Kuhlmann & Hazelton, 2007), although dispersal rates rarely exceed 3 km/year. In comparison, the rate of spread by rusty crayfish in the JDR is much higher, averaging more than 18 km/year for the period 2005 - 2016 (Sorenson *et al.*, 2012; *this study*). Such high rates of dispersal, however, are not unprecedented; spiny-cheek crayfish *Orconectes limosus* spread 13-16 km/year in the Danube River (Puky & Schád, 2006), and signal crayfish *Pacifastacus leniusculus* dispersed at a rate of 18-24 km/year in the Drava River, Croatia (Hudina, Faller, Lucić, Klobučar, & Maguire 2009). Given the clear differences in literature-based vs. observed dispersal rates for rusty crayfish, the monthly dispersal rate of rusty crayfish for this model was determined through inverse modeling and calibration. In other words, parameter values of dispersal were adjusted to match observed crayfish distributions, according to principles of pattern-oriented modeling.

1.11.4.9 Factors affecting rate of spread

Many factors can affect the rate of spread of crayfish and could explain the discrepancies between short and long term observations, as well as differences in invasion speed among systems. Overall, the spread rate of rusty crayfish is influenced by two main demographic processes: dispersal and population growth (Gallien, Münkemüller, Albert, Boulangeat, & Thuiller 2010), both influenced by similar factors. Indeed, while temperature and water velocity are the main environmental factors physically influencing movement speed of rusty crayfish, other factors such as availability of cobble habitat (Kershner & Lodge, 1995; Perry *et al.*, 2001), predation pressure [predation risk highly decreases crayfish activity (Butler & Stein, 1985; Hill & Lodge, 1994)], and crayfish density are also likely to heavily influence their speed and rate of success when dispersing due to their impact on behavior and survival. Another factor that might explain discrepancies between short-term observed movements and long scale spread is the difference between exploratory movement and movement related to homing. In comparing movement kinematics between outward and homeward movement of rusty crayfish, Kamran and Moore (2015) showed that homeward movement was much faster than outward movement due to both increased walking speed and decreased turning angles in the former type of movement. This could suggest that exploratory movement in long distance movement would be slower than that observed over a few days in many mark-recapture experiments and laboratory observations.

Water velocity

At high water velocity, crayfish are at risk of dislodgment from the channel bed and drift entry despite their ability to modify their position in swift current. In fast flowing waters, crayfish flatten their body against bed sediment, lowering their telson, and grasp sediment with their chelae (Maude & Williams, 1983; Clark, Kershner, & Holomuzki 2008), but at high enough water velocities, movement is brought to a halt as crayfish seek refuge (Clark *et al.*, 2008). Prins (1968) observed that during spring floods, rusty crayfish of all sizes sought refuge in vegetation along banks and in eddies. Smaller specimens are thought to be more sheltered from the effects of fast flow as they can more easily exploit interstices among bed sediments (Prins 1968; Clark *et al.*, 2008) but larger crayfish are able to sustain higher velocities in the absence of shelter (Foster & Keller, 2011), and differences between sexual forms do not influence slip speed (Maude & Williams, 1983). In a study of signal crayfish *Pacifastacus leniusculus* in upland rivers of England, Bubb *et al.* (2004) report that no large scale movement (> 20 m) occurred during periods of high flow and that the proportion of crayfish moving was negatively correlated to flow.

In Ozark streams, studying *Orconectes* species (not including *Orconectes rusticus*), Flinders and Magoulick (2007) noted that current velocity was a significant variable in determining crayfish density. Orconectes species selected habitats with reduced flow, which likely reduces the rate of accidental drift, and may increase foraging efficiency and reduce predation risk. In laboratory experiments, Maude and Williams (1983) showed that, overall, rusty crayfish ranks high among Orconectid species in its ability to withstand swift current. Not only was rusty crayfish collected at sites downstream of dams with surface velocity exceeding 1 m/s on a regular basis, but their mean slip speed (the average speed at which crayfish started slipping) on Plexiglas in the laboratory was 40.2 cm/s (95%CI: 36.6 – 43.8 cm/s). Similarly, mean impedance velocity (speed at which crayfish stopped moving) for rusty crayfish in culverts was 7.7 cm/s (24%) higher than *O. virilis* and 5.6 cm/s (16.5%) higher than *O. propinquus*, with 75% of rusty crayfish impeded by water current of 44.5 cm/s (Foster & Keller, 2011). In laboratory settings, Clark *et al.* (2008) showed that *O. robustus* were dislodged twice as often when moving downstream than upstream, such that 65% of crayfish moved upstream at increased current speed. They also observed that small and medium *O. obscurus* could occupy riffles during floods even when current velocity exceeds 1 m/s, whereas larger crayfish need to burrow, find shelter in substrate, or move to backwaters. Activity of *O. obscurus* is greatly reduced at 0.8 m/s with virtually no activity beyond 1 m/s, even in cobble habitat, and most medium and large crayfish are dislodged at water velocities between 0.6 m/s and 1 m/s in small pebble and small cobble habitat, respectively (Clark *et al.*, 2008). These latter findings contrast with average dislodgment velocities of 0.3 m/s reported by (Maude & Williams, 1983) for similarly-sized *O. obscurus* on exposed, roughened Plexiglas.

Water velocity also has a long term effect on rusty crayfish phenotype. Indeed, in agreement with the observation that younger crayfish tend to be found in faster flowing waters (Prins, 1968; Clark *et al.*, 2008), the size and shape of chelae and bodies of rusty crayfish differ among populations depending on stream water velocities. In high-velocity streams, crayfish have smaller bodies and shorter, broader chelae than crayfish in low velocity streams and lakes (Perry *et al.*, 2013). Only a few individuals over 38 mm and no crayfish over 45 mm CL were found in high velocity streams while individuals over 40 mm are a common occurrence in the literature on lakes crayfish (Perry *et al.*, 2013).

High water velocities and recurrent floods might even completely prevent establishment of rusty crayfish in some areas. Bobeldyk and Lamberti (2008) hypothesized that the absence of rusty crayfish in the Middle Branch of the Ontonagon River downstream of Bond Falls is likely due to hydrologic disturbance resulting in reduced mobility, recruitment, foraging, and potentially leading to dislodgement and mortality. They point out the absence of reproductively active or gravid females in the fast flowing reaches, a phenomenon also observed by Sorenson (2012) in the JDR, who observed very few crayfish and only one gravid females following high flows in spring.

Due to these limitations on sustained water velocity, crayfish might also be limited in their spread by culvert. Based on observed impedance velocities, Foster and Keller (2011) determined that 20% of the culverts their studied in Michigan were impassable to rusty crayfish even during summer low-flow conditions. Similarly, dams and weirs are thought to limit the upstream and sometimes downstream spread of rusty crayfish. Rusty crayfish was likely limited in its downstream dispersal by dams and weirs in the Speed River in Ontario (Reid & Nocera, 2015). Keller and Hazlett (2010) observed rusty crayfish populations upstream of Burt Lake in the Maple River, Michigan all the way, but no crayfish were found further upstream past the dam. A flood control weird and a large waterfalls (7 m) have also been observed to stop the advance of rusty crayfish upstream, while beaver dams have largely slowed their downstream progression (Momot, 1996).

While high water velocity and subsequent dislodgement is known to often cause direct mortality in crayfish, little information is available on the eventuality of crayfish passively drifting downstream and surviving. Passive downstream dispersal is relatively common in other stream invertebrates Wubs *et al.* (2016), but there is no evidence of such phenomenon in crayfish, though we believe that passive dispersal of juveniles would not largely differ from that of other macroinvertebrates.

Depth

The impact of depth on crayfish movement is not well documented in rusty crayfish despite a wealth of information on lake populations where crayfish are most often found in the littoral zone under 8 m depth (Hein, Vander Zanden, & Magnuson 2007; Jansen *et al.*, 2009) but can move to deep waters during the winter (high densities at 9 m depths; Claramunt, Jonas, Fitzsimons, & Marsden 2005) and occur at 14.6 m in Lake Michigan (Taylor & Redmer, 1996). We hypothesize that depth is unlikely to be a limiting factor of rusty crayfish spread in the JDR, but it could limit its distribution to the littoral areas of the Columbia River.

Temperature

As a poikilotherm, rusty crayfish can tolerate a wide range of conditions yet their metabolic rates are highly dependent on water temperature. Ambient temperature affects crayfish dispersal speed through metabolism. The few studies that tracked the influence of water temperature on crayfish movement in the field were done at small scales, small sample sizes, and with a limited temperature range.

While rusty crayfish can remain locally active at very low temperatures, large-scale movement is likely to be highly diminished at a higher temperature threshold. A number of studies have shown that growth and survival rates are parabolic from 0°C and 40°C with a thermal optimum between 20°C and 25°C (Claussen, 1980; Mundahl, 1989; Mundahl & Benton, 1990; Ellrott *et al.*, 2007; Keller & Hazlett, 2010). Therefore, although rusty crayfish can remain locally active at low temperatures, large-scale movement is rare. Prins (1968) reported a decrease in rusty crayfish activity below 6-8°C and complete torpor at 4°C and Hamr (1997) found that rusty crayfish were immobile between 0 and 6°C. Bubb, Lucas, and Thom (2002) observed in *Pacifastacus leniusculus* that crayfish might remain slightly active at lower temperatures with a linear temperature-mediated response (with activity levels falling with decreasing temperature) at a temperature range of 1-8°C but are entirely inactive in terms of large scale movements. They observed a complete halt of large-scale movements when temperature dropped from 8°C to 4°C in December. In another study on the same river, Bubb *et al.* (2004) showed that there was a significant positive relationship between mean water temperature and range per day tracked ($R^2 = 0.24$) with range/day going from nearly 0 at 4°C to ranges commonly over 2 m/day and sometimes as high as 14 m/day at temperatures 12-16°C for signal crayfish. However, in both studies, the authors caution of the low temperatures in which activity was recorded. The range of movements recorded are believed to be those found at relatively low temperatures and might even be smaller scale movements.

In *O. limosus*, Buric, Kouba, and Kozak (2009) observed large scale movements occurring largely during mating with no upstream or downstream bias (14.9 m per day on average for males), and only small movement distances after the end of mating (2.2 m per day on average). However, during mating, there was no correlation between water temperature and movement while movement distances closely followed water temperatures after the mating (Spearman rank correlation rs = 0.477).

Using quantile regressions, Johnson, Rice, and Reid (2014) found that the median regression predicted a cessation of activity for signal crayfish below 5°C and an increase in daily-averaged distanced moved of 2.5 m/°C. However, their study did not include a period for which temperature decreased below 5°C. Moreover, they only observed local movements within a reach 20 m-long noting that crayfish on average stayed in the reach for 11 days (SD = 9 days) before leaving and rarely returning.

Comparison of movement data between studies on different species is difficult. In a laboratory experiment, Lozán (2000) shows that while the movement activity vary similarly according to temperature across four species (*A. leptodactylus, O. limosus, A. astacus, P. leniusculus*) with highest levels of activity recorded at 20°C and decreased activity at higher and low temperature (lowest amount of activity at 4 degrees), their values widely differ. Similarly, the recorded movement speed and daily covered distanced at 20°C of *P. leniusculus* (mean 47.22 cm/min and 190 m/day) is many times inferior to that of *O. limosus* (134.83 cm/min and 664 m/day) and *A. leptodactylus* (285.25 cm/min and 1587 m/day).

Based on these studies, the model assumed that movement distance of each crayfish was linearly dependent on the proportion of days each month where average daily water temperature exceeded 6°C. This calculation was performed at the reach scale. For example, if average daily water temperature > 6°C for 15 days in a month, crayfish were moved half the distance relative to what is possible if all days exceed 6°C. If a crayfish reached a confluence where one tributary’s water temperature never exceeded 6°C for the entire month, it would disperse to the other tributary with a probability of 0.8 as long as that reach had at least one day above 6°C.

1.11.5 Appendix 1.5: dispersal kernel recalibration after 2016 survey

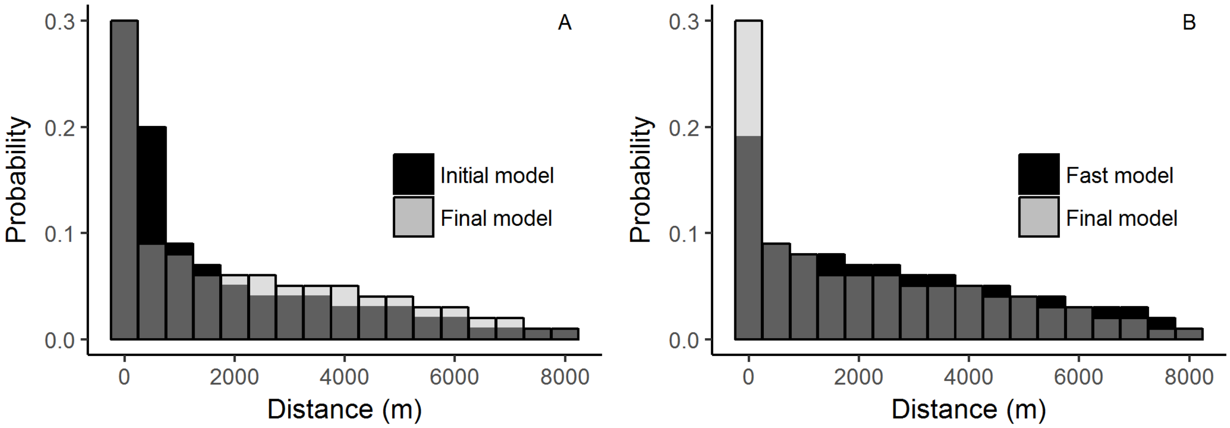

Figure 1.S3. Dispersal kernel (probability distribution) used in determining the monthly distance moved by rusty crayfish in (A) the initial and final model (light grey), and (B) in a sensitivity analysis comparing a more leptokurtic kernel to the final model. Each month in the model, each crayfish draws the distance that it will move from 17 possible distances in 500-m increments based on these probability distribution. The final model is more leptokurtic than the initial model due to a decrease in the probability of short distance movements and an increase in the probability of long distance movements. For both models in (A), 30% of crayfish do not move every month. In the fast model (B), only 19% of the crayfish population does not move.

1.11.6 Appendix 1.6: comparison of observed and modeled rusty crayfish densities

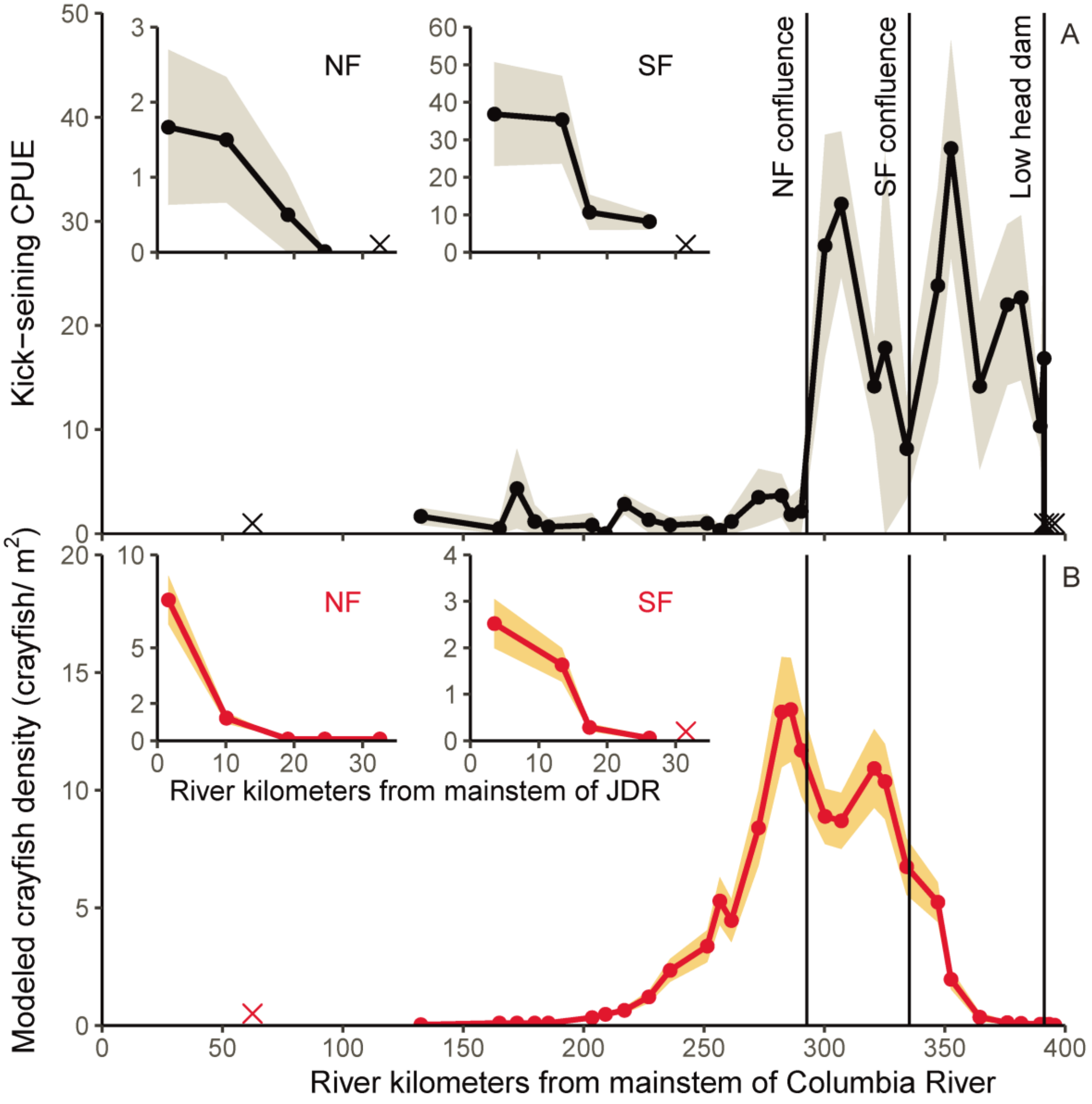

Figure 1.S4. Longitudinal profile of (A) the observed relative density and (B) HexSim model density of rusty crayfish in the JDR basin in August 2016. The observed relative density (y-axis in A) is the mean ± SD catch-per-unit-effort (CPUE) from six 1-m^2 area-standardized kick-seines (see Methods) while the modeled density (y-axis in B) is the mean ± SD number of crayfish in each reach from 10 replicated runs of the HexSim model divided by the estimated wetted width (see Appendix 1.1). Points represent surveyed sites where rusty crayfish were either observed (A) or modeled (B) present in August 2016 whereas crosses represent sites where crayfish were observed or modeled absent. In the main stem of the JDR (main panels), modeled densities generally resemble observed CPUE, though core population areas with the highest densities are predicted further downstream than observed during the survey. In the North Fork of the JDR (NF, left-hand insets), modeled densities exceed observed CPUE while in the South Fork (SF,

right-hand insets), modeled densities are far inferior to observed CPUE. Differences between 1-m^2 kick-seining CPUE and modeled densities in the main stem JDR could be due to the use of comparatively large wetted width estimates to compute modeled densities (August 2016 discharge in the JDR was about 4 times lower than historical median discharge). In addition, kick-seining was conducted on optimal crayfish habitat representing a subset of the channel while modeled densities were calculated for the entire reach area.

1.11.7 Appendix 1.7: parameters and results of the final model and sensitivity analysis

Table 1.S4. Parameters and results of the final model and of the sensitivity analysis. All model parameters are in bold. Results are not. Dashes show parameters for which no additional values than the initial "normative" values were tested. Results for August of 2005, 2010, and 2016 (in this order), include the mean of the estimated rusty crayfish population size (N), in thousands of individuals including males and females, and of the total length of river invaded by rusty crayfish (in kilometers), over 10 replicate runs for the final model and the percentage departure from these values for all other models in the sensitivity analysis. We conducted a local sensitivity analysis, such that each parameter value was changed one at a time while keeping the others fixed. For instance, for the lowest value of the number of initial propagules, only the number of propagules was changed and set to 2 whereas the carrying capacity was kept at 15.

Event	**Parameter** Output	**Final model**	**Lowest: -75%**	**Low: -20%**	**High: +20%**	**Highest: +100%**
			% change from final model in mean population size | invaded extent			
	Initial propagules (# of females)	**10**	**2**	**8**	**12**	**20**
	08/2005 – N | spread	27.1 | 200	-85 | -56	-11 | -2	+35 | +8	+121 | +20
	08/2010 – N | spread	919 | 423	-86 | -45	-10 | -5	+32 | +3	+115 | +10
	08/2016 – N | spread	48297 | 705	-82 | -30	-9 | -2	+23 | +4	+63 | +9
Reprod. status	**% females that are reproductive**					
	age 1	**10**	-	-	-	-
	age 2	**80**	-	-	-	-
	age 3	**90**	-	-	-	-
Reproduction	**Timing**	**July**	-	-	-	-
	Mean intrinsic fecundity (SD)					
	age 1|age 2|age 3	**80(10)|120(20)|150(40)**	**20|30|37.5**	**64|96|120**	**96|144|180**	**160|240|300**
	08/2005 – N | spread	27.1 | 200	-99 | -78	-47 | -13	+180 | +22	+1972 | +52
	08/2010 – N | spread	919 | 423	-100 | -88	-71 | -18	+325 | +15	+6879 | +52
	08/2016 – N | spread	48297 | 705	-100 | -93	-81 | -17	+190 | +18	+1683 | +66
	Carrying capacity	**15**	**4**	**12**	**18**	**30**
	08/2005 – N | spread	27.1 | 200	+6 | +4	0 | 0	0 | 0	-6 | -3
	08/2010 – N | spread	919 | 423	-1 | -3	-4 | -1	-3 | -4	-7 | -6
	08/2016 – N | spread	48297 | 705	-45 | 0	-9 | +2	0 | -3	+7 | -3
Survival	**Yearly survival (%)**					
	age 0|age 1|age 2|age 3	**8|70|45|2**	**2|-|-|-**	**6.5|-|-|-**	**9.5|-|-|-**	**16|-|-|-**
	08/2005 – N | spread	27.1 | 200	-96 | -70	-47 | -17	+73 | +9	+1013 | +58
	08/2010 – N | spread	919 | 423	-100 | -82	-68 | -19	+142 | +11	+3498 | +51
	08/2016 – N | spread	48297 | 705	-100 | -85	-77 | -19	+111 | +14	+432 | +64
Movement	**Movement**	**Figure S3 (light grey)**	**Constant**	**Figure S3A (black)**	**Figure S3B (black)**	**Uniform**
	08/2005 – N | spread	27.1 | 200	+28 | -30	-4 | -10	+6 | +6	+15 | +16
	08/2010 – N | spread	919 | 423	+27 | -46	-4 | -15	+2 | +5	+12 | +18
	08/2016 – N | spread	48297 | 705	+8 | -46	-18 | -12	+7 | +6	+23 | +22
	Movement direction (upstream/downstream)	**50/50**	-	-	-	-
	Influence of temperature	**Days > 6°C**	**None**		**Parabolic**	
	08/2005 – N | spread	27.1 | 200	+8 | +2		-1 | +2	
	08/2010 – N | spread	919 | 423	+5 | +1		-2 | +0	
	08/2016 – N | spread	48297 | 705	+5 | +3		-9 | +5	
	Upstream resistance	**% dispersal distance upstream relative to downstream movement**				
	Gradient: 0.001-1%	**0.375**	**0**	**0.25**	**0.5**	**1**
	Gradient: 1-5%	**0.1875**	**0**	**0.125**	**0.25**	**1**
	Gradient: 5-10%	**0.075**	**0**	**0.05**	**0.1**	**1**
	Gradient: 10-30%	**0**	**0**	**0**	**0**	**1**
	08/2005 – N | spread	27.1 | 200	+4 | -46	+5 | -13	+10 | +13	+21 | +69
	08/2010 – N | spread	919 | 423	+4 | -55	+4 | -26	+7 | +13	+15 | +104
	08/2016 – N | spread	48297 | 705	+10 | -60	+7 | -25	-7 | +23	-36 | +137

1.11.8 References

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Chapter 2

Phenotypic variability of rusty crayfish (*Orconectes rusticus*) at the leading edge of its riverine invasion

Mathis L. Messager[1, 2], Julian D. Olden[1]

[1]*School of Aquatic and Fishery Sciences, University of Washington, Seattle, Washington, USA 98195*

[2]E-mail: messamat@uw.edu

2.1 Abstract

Species around the globe are undergoing phenotypic shifts at ecologically relevant timescales as they invade new ecosystems and track changing temperatures. Disentangling the contribution of environmental gradients from range expansion itself in driving these changes and identifying the specific traits undergoing selection is crucial to predict the future spread and impact of species on the move. Here, we investigate phenotypic changes in rusty crayfish *Orconectes rusticus* (now *Faxonius rusticus*), an ecologically important invasive species, through an extensive survey of their invasion gradient in multiple tributaries of the John Day River (JDR, Oregon, U.SA.), a major tributary of the Columbia River. Rusty crayfish in the JDR have developed less competitive morphology (lighter body and smaller claws) but better physiological condition (intrinsic growth and/or reproductive potential measured as RNA/DNA ratio) as they spread towards the edge of their current invasion range, and feed lower in the food web in invasion front populations than in core areas. By accounting for variations in temperature, primary productivity, and macroinvertebrate biomass throughout the invasion extent of rusty crayfish, our findings suggest that low conspecific densities at the invasion edge and spatial sorting primarily drive these phenotypic changes. These trends are thus likely to grow stronger over time as rusty crayfish keeps spreading towards the Columbia River.

2.2 Keywords

Aquatic invasive species, *Faxonius rusticus*, invasive crayfish, John Day River, leading edge, *Orconectes rusticus*, range margin, RNA/DNA, rusty crayfish, spatial sorting, trophic position, secondary spread

2.3 Introduction

Species around the globe are exposed to changing selection pressures as they invade new landscapes or shift their range to track climate change (Moran & Alexander 2014). As their range shifts and expands, the individuals in the vanguard of these populations often find themselves exposed to novel environmental conditions, predators, and competitors. Mounting evidence suggests that these factors, in combination with low conspecific densities relative to those experienced by core populations, promote rapid changes in species phenotypes at range boundaries (Chuang & Peterson 2016).

Phenotypic changes at the leading range edge of invasive populations have been observed in a number animals, including amphibians [e.g. cane toad *Rhinella marina* (Perkins et al. 2013)], insects [e.g. ground beetle *Merizodus soledadinus* (Laparie et al. 2013)], fish [e.g. round goby *Neogobius melanostomus* (Brandner et al. 2013)] and decapods [e.g. signal crayfish *Pacifastacus leniusculus* (Hudina et al. 2012)], and affect traits ranging from body length and fecundity to boldness (Chuang & Peterson 2016). Such changes have been associated with accelerating invasion rates (Burton, Phillips & Travis 2010; Phillips, Brown & Shine 2010a; Weiss-Lehman, Hufbauer & Melbourne 2017) and increased impacts to recipient ecosystems (Brandner *et al.* 2013; Iacarella, Dick & Ricciardi 2015). Our ability to predict the distribution of range shifting species has long been challenged by these departures from the equilibrium dynamics characterizing species with a stable range, and the stochastic effect of serial founder events and drift on range boundaries (Elith, Kearney & Phillips 2010; Phillips 2015). Enhanced insight into the processes leading to phenotypic changes in invasive species is thus essential for anticipating their future spread and impact, as well as predicting the outcome of species range shifts in response to climate change (Caplat *et al.* 2013; Travis *et al.* 2013).

Phenotypic plasticity (Davidson, Jennions & Nicotra 2011), natural selection (Brown, Kelehear & Shine 2013), and spatial sorting (Shine, Brown & Phillips 2011) are the three main processes responsible for observed trait variability at range edges, yet their respective contributions are seldom understood. Phenotypic plasticity, the ability for multiple phenotypes to arise from a single genotype in response to changing environmental conditions, is particularly prevalent in invasive species (Davidson, Jennions & Nicotra 2011). It is crucial in allowing populations to spread and adapt to changing environments faster than would otherwise be possible by evolution through natural selection alone (Chevin, Lande & Mace 2010). Furthermore, abiotic and biotic differences at the invasion front can lead to trait evolution by natural selection. Low intra-specific densities in particular are likely to shift selective pressures towards higher growth and reproduction (Phillips, Brown & Shine 2010b). Lastly, the range expansion process itself can lead to adaptive changes in traits through spatial sorting, whereby the fastest dispersing individuals at the expanding edge of the population systematically interbreed, resulting in selection for enhanced dispersal ability in their offspring if dispersive traits are heritable (Shine, Brown & Phillips 2011). This runaway process continues in subsequent generations until trade-offs between traits begin to limit the potential for directional selection (Burton, Phillips & Travis 2010). The traits involved in promoting dispersal and growth at the invasion front are so numerous that natural selection and spatial sorting can impact morphology, physiology, behavior, immunology, and life-history, among others (Chuang & Peterson 2016). Understanding the specific traits undergoing selection in invasive species and disentangling the influence of environmental conditions from contemporary evolution therefore requires empirical studies that are specific to the species and systems at hand.

Riverine ecosystems are ideal to study the eco-evolutionary dynamics unfolding at range boundaries. In these systems, obligate freshwater species spread upstream and downstream along largely monotonic gradients in environmental conditions (e.g. lower slope, wider and deeper channels, and warmer waters downstream; Mari *et al.* 2014). The monotony and hence predictability of these gradients could enable ecologists to better account for the influence of plasticity to environmental conditions when studying phenotypic changes at range boundaries. When considering temperature for instance, higher growth rates observed in both the upstream and downstream invasion fronts of an ectotherm invasive species would suggest that growth-enhancing traits are being selected in dispersing individuals despite temperatures increasing towards the downstream front and decreasing upstream, *ceteris paribus*.

Crayfish are among the most widely introduced freshwater animals worldwide (Lodge *et al.* 2012). Following their introduction, nonnative crayfish can cause severe ecological impacts across entire food webs because they can reach extremely high local densities and display polytrophic feeding habits (Twardochleb, Olden & Larson 2013). In invaded ecosystems, native crayfish species can be displaced within a few years, and populations of macrophytes, insects, snails, and fish often decline (McCarthy *et al.* 2006; Olden *et al.* 2006; Rosenthal, Stevens & Lodge 2006; Bobeldyk & Lamberti 2010). Changes in population structure, behavior, morphology, and physiology have already been reported between core and edge populations in several ongoing river invasions by crayfish (Hudina *et al.* 2012; Hudina, Zganec & Hock 2015; Pârvulescu *et al.* 2015; Rebrina *et al.* 2015). Even though the consequences of these changes on invaded ecosystems remain unexplored, increased invasion rates alone could challenge our ability to respond to new and ongoing crayfish invasions. In addition, given that the impact of an invasive species on the recipient ecosystem is not only a function of its range size but also of its abundance and per-capita effect (Parker *et al.* 1999), changes in somatic and reproductive growth rates or trophic niche could have severe consequences for native communities. A better grasp of the potential phenotypic shifts occurring at the front of crayfish invasions could thus shed light on both the selection pressures exerted upon dispersing populations in general and on the future impact of these invasions.

Objectives

In this study, we investigated changes in rusty crayfish [*Orconectes rusticus,* Girard 1952, now *Faxonius rusticus* (Crandall & De Grave 2017)] traits across their invasion gradient in the John Day River (JDR) by addressing the following two questions. First, are there phenotypic differences in rusty crayfish individuals progressing from established core populations near the initial location of their introduction to recently colonized invasion fronts? We used a riverscape survey to analyze rusty crayfish population structure, morphology, physiological condition, and trophic position across its range in the mainstem of the JDR and its main tributaries. We hypothesized that changes in phenotypes across rusty crayfish generations occurred as they dispersed from their location of introduction to their present invasion fronts in the JDR. We expected that low conspecific densities in newly invaded river sections would lead to increased access to resources and relative consumption of growth-inducing food like macroinvertebrates (Hill, Sinars & Lodge 1993). We thus expected that rusty crayfish would exhibit better physiological condition and higher trophic position towards the invasion front and, as a result, larger carapace length and higher weight (Brown, Kelehear & Shine 2013). We also posited that two additional mechanisms could affect crayfish traits during range expansion. If larger, faster growing, and more competitive crayfish are better dispersers, as has been reported in multiple invasions (Chuang & Peterson 2016), we expected to observe an increase in crayfish body size, physiological condition, and relative chela length towards the invasion front. By contrast, if trade-offs exist among crayfish traits, then selection for high population growth rates and faster dispersal at the invasion leading edge could lead to unexpected changes in other traits (e.g. decreased chela length) towards the invasion front (Phillips, Brown & Shine 2010).

Second, are these phenotypic changes in populations towards the invasion front caused by plasticity to environmental factors or selection due to range expansion? We sought to disentangle these two sets of processes by studying rusty crayfish subpopulations both upstream and downstream of their initial location of introduction and by simultaneously accounting at each site for the distance from the invasion core (reflecting the invasion stage at that location), gradients in environmental conditions, and the availability of food resources. We hypothesized that if selection due to the range expansion process was driving changes in rusty crayfish traits as they spread throughout the JDR, then these phenotypic differences would become larger in sites further from the core and be greatest at the invasion fronts, regardless of whether populations spread upstream or downstream and the local gradient in environmental conditions.

2.4 Data and methods

2.4.1 Study area

The JDR originates in the Blue Mountains of northeastern Oregon (U.S.A) and flows undammed for 457 km until it enters the Columbia River just upstream from the Columbia River Gorges (Fig. 2.1). One of the largest free-flowing rivers in the United States with a drainage area of 21,000 km^2, the JDR is of high conservation importance as it supports several fish species of high cultural and economic value, including endangered spring Chinook salmon, *Oncorhynchus tshawytscha,* and threatened steelhead, *O. mykiss*.

Rusty crayfish was first found in the JDR in 2005, marking its first recorded occurrence west of the North American continental divide (Olden, Adams & Larson 2009). Evidence suggests that rusty crayfish were first released in the late 1990s in the mainstem JDR, about 380 river kilometers upstream from its confluence with the Columbia River, near the town of Mount Vernon, Oregon, by a teacher and students of a nearby school (Olden, Adams & Larson 2009). In the ~20 years since their presumed date of introduction, rusty crayfish rapidly spread throughout the JDR watershed at rates exceeding 15 km/year, raising concerns that the mainstem of the Columbia River may soon be reached (Olden, Adams & Larson 2009; Sorenson, Bollens & Counihan 2012; Chapter 1). Only the native signal crayfish *Pacifastacus leniusculus* was known to be present in the watershed prior to the introduction of rusty crayfish in the John Day River (Larson & Olden 2011).

2.4.2 Field survey site selection and crayfish sampling protocol

We implemented a spatially extensive survey of rusty crayfish relative densities, phenotypes, and environmental conditions throughout its invasion range to capture gradients in these variables from core to leading-edge populations. The secondary spread of rusty crayfish in the JDR watershed was first simulated from the putative date and location of initial crayfish introduction to present day with a spatially explicit individual-based model using data on the historical distribution of rusty crayfish and environmental conditions in the JDR (Chapter 1). We then used model predictions for August 2016 to distribute 60 sampling sites every 5-10 km along the main stem and primary tributaries of the JDR, encompassing the invasion extent of rusty crayfish. Sampling was conducted August 1–22, 2016.

To assess the relative density of rusty crayfish across the watershed, area-standardized kick-seining was performed in six locations across a 50-m long reach at each surveyed site. One person disturbed 1 m^2 of substrate upstream of a seine net held by another team member to flush crayfish downstream, yielding a mean and standard deviation of crayfish density at each site. To ensure consistency in our measure of relative density, we exclusively sampled in runs (i.e., rather than pools or riffles) when possible, because runs provide the water velocity and depth needed for this sampling method to be most effective (Larson & Olden 2016). To avoid false absences, snorkeling, hand-netting, and baited traps were also used when rusty crayfish were not detected using seining.

Where rusty crayfish were found, the sex, carapace length (mm), chela length (mm), weight (g), missing chelae (yes/no), and molting condition (yes/no) of captured crayfish were measured at every site, while two tissue samples (abdominal white muscle) from 14 rusty crayfish were taken at every other site. When our standard sampling protocol yielded less than 14 crayfish, additional specimens were caught by hand-netting so that these measurements and tissue samples could be taken — though these individuals were not included in our estimates of relative density. On the contrary, where the crayfish population was dense, morphological measurements were recorded for a random subsample of 30 of the crayfish that were caught by kick-seining. The first tissue sample was immediately stored in non-iodized salt for subsequent $\delta^{15}N$ stable isotope analysis to determine the trophic position of rusty crayfish at that site — the energy-weighted number of trophic energy transfers from primary producer to crayfish (Vander Zanden & Rasmussen 1999). We also collected 12–20 mayfly nymphs (Ephemeroptera: Heptageniidae) in runs and riffles at each study site where crayfish tissues were sampled to characterize the $\delta^{15}N$ values of primary consumers at each site throughout the JDR (Anderson & Cabana 2007). The second tissue sample, preserved in RNA*later*®, was used to quantify the relative concentration of RNA and DNA in rusty crayfish cells. While the amount of DNA remains mostly constant in cells regardless of conditions, the amount of RNA positively correlates with the amount of protein synthesis (anabolic activity). Therefore, the ratio of the amount of RNA to that of DNA in a cell is an effective eco-physiological indicator of condition (hereafter 'physiological condition') that reflects the organism's potential investment in somatic growth and gamete production (i.e. fertility) under a given set of environmental conditions (Koop *et al.* 2011).

Environmental conditions at each site were characterized by measuring water depth, temperature, and velocity, as well as benthic chlorophyll *a* concentration of green algae and diatoms at 10 points along a transect perpendicular to the river banks. The benthic concentration of chlorophyll *a* is a proxy of benthic algal biomass (Dodds, Smith & Lohman 2002) measured using a Benthotorch (Kahlert & McKie 2014). The biomass of macroinvertebrates was also quantified at all sites where crayfish tissue samples were taken. The abundance of macroinvertebrates was assessed by taking three 0.09-m^2 (1-ft^2) standardized samples in runs and riffles with a D-frame kick net. All macroinvertebrate samples were then washed through 0.5 mm sieves and preserved in 70% ethanol.

2.4.3 Stable isotope analysis

Stable isotope analysis was conducted on rusty crayfish tissues and mayfly whole specimens to assess differences in crayfish feeding patterns throughout their invasion gradient. All samples were prepared for isotope analysis using standard protocols, with the exception of the preservation method. Preservation in non-iodized salt is an easy and inexpensive technique, when freezing is unpractical due to remote field conditions, and results in minimal and directionally uniform effects on $\delta^{13}C$ and $\delta^{15}N$ (Arrington & Winemiller 2002). Prior to processing, all crayfish muscle tissues and mayfly whole bodies were extensively rinsed with distilled water until all the salt was dissolved. Samples were then dried at 60°C for 24 h. Once dry, samples were ground to powder for homogenization using a stainless steel mortar and pestle, of which approximately 1 mg (± 0.2 mg) was placed in tin capsules and sent for analysis with an Isotope Ratio Mass Spectrometer (IRMS) to the University of California Davis Stable Isotope Facility. The trophic position of each crayfish at site (S) was estimated according to:

$$Trophic\ position_{crayfish,S} = 2 + \frac{\delta^{15}N_{crayfish,S} - \left(\sum_{mayfly=1}^{3} \delta^{15}N_{mayfly,S}\right)/3}{2.54}, \tag{1}$$

where 2.54 is the rusty crayfish discrimination factor or fractionation factor (Δ) representing the absolute difference in δ^{15}N between rusty crayfish and its diet, determined in laboratory based on an algae diet (Glon, Larson & Pangle 2015). We applied a single fractionation factor to all trophic links of the food web between primary consumers and crayfish and thus did not account for fractionation differences among crayfish diets due to a lack of more specific reference values.

2.4.4 RNA-DNA analysis

The procedure for the extraction and quantitation of nucleic acids in rusty crayfish tissues was adapted from Berdalet *et al.* (2005) and Vrede, Persson and Aronsen (2002) using fluorochromes that indiscriminately bind to DNA and RNA (see Appendix 2.1 for details on reagents). Following storage of the crayfish tissue samples in RNA*later* on ice for up to 10 days, supernatant RNA*later* was removed from vials and samples were frozen at -80 °C until nucleic acid extraction.

Both mechanical and chemical methods were used in extracting nucleic acid from crayfish tissues to maximize homogenization. For each sample, after slightly thawing the tissue at 0-4°C, 200 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) was added to the vial and the sample was crushed for one minute. The head of the pestle was then cut off with a pair of nippers and left in the vial. To chemically extract nucleic acids, the sample was shaken in a multi-head vortex at room temperature for 60 minutes with the pestle head and 200 µl of STEB buffer (1% w/v solution of N-Lauroylsarcosine sodium salt in TE buffer). The sample was then centrifuged at 10,000 rpm for two minutes to pellet cell and filter debris. Lastly, 100 µl of supernatant homogenate was transferred to another vial and stored frozen at -80°C until further analysis. We measured the amount of extracted RNA and DNA in crayfish tissue samples based on the method developed by Berdalet *et al.* (2005) and Berdalet, Roldán and Olivar (2005) adapted to use the Quant-iT™ RiboGreen® RNA Assay Kit with 96-well microtiter plates. Berdalet, Roldán and Olivar (2005) recommend using three separate aliquots of each sample to compute the quantity of RNA and DNA in crustacean tissues: the first measures RNA after DNA digestion; the second measures DNA after RNA digestion; and the third measures residual fluorescence after digestion of both DNA and RNA. Nuclease working solutions also included calcium chloride and magnesium chloride to optimize digestion. After being thawed, mixed, and centrifuged, the 100 µl of isolated sample homogenate was diluted (1:10) in Tris buffer and three 40-µl aliquots were transferred to separate vials to run the DNase, RNase, and residual assays. We then incubated aliquots with nucleases at 37°C for 90 min, diluted them in 460 µl of TE buffer, and transferred 50 µl of the resulting solution in the microtiter plate wells. Four nucleic acid standard curves with six concentrations each were run for every plate of samples: RNA+DNase, RNA+RNase, DNA+RNase, and DNA+DNase. The standard solutions were processed identically to sample assays, including the addition of salts and STEB extraction buffer in the same concentrations as in the sample assays so as to control for extraneous sources of fluorescence. Two replicates for every sample aliquot and standard curve solution were included in the plate, and samples from multiple non-adjacent sites were used in each plate to avoid systematic biases due to plate-specific variations in measurements. To quantify RNA and DNA fluorescence, 50 µl of diluted (1:200) Quant-iT™ RiboGreen® reagent was added to each plate well. The slope of the standard curves were then estimated using linear regression and the quantity of RNA [µg RNA/ml assay], DNA [µg DNA/ml assay], and their ratio in each sample was calculated using the equations provided by Berdalet *et al.* (2005).

2.4.5 Macroinvertebrate biomass

Macroinvertebrate Ash Free Dry Weight (AFDW) was estimated at every other site to reflect the prey biomass available for consumption by rusty crayfish, one of the main environmental drivers of crayfish trophic position (Olsson *et al.* 2008). Macroinvertebrates were sorted using a stereo microscope and separated from other material (sand, algae, etc.) found in the samples. Sorted macroinvertebrates were then rinsed with distilled water and dried in an oven at 60°C for 48 h, weighed, combusted in a muffle furnace at 550°C for four hours (Mason 1983), cooled down to room temperature in a desiccator for 6 h, and reweighed. Ash mass (after combustion in furnace) was then subtracted from dry mass (before combustion) to obtain AFDW.

2.4.6 Data analysis

The goal of this study was to assess whether a phenotypic shift has occurred along the invasion gradient of rusty crayfish, and whether this shift was driven by the range expansion process or by longitudinal gradients in environmental conditions. Environmental conditions and the speed of rusty crayfish spread differed among tributaries, so each invasion leading edge was analyzed separately. In total, four leading-edge populations were analyzed, one downstream edge in the mainstem JDR and three upstream edges — in the mainstem, South Fork, and North Fork

JDR (Fig. 2.1). The mean annual discharge of the North Fork JDR is about twice that of the mainstem JDR at their confluence, such that a discontinuity could be expected between the lower mainstem (past the confluence of the North Fork and mainstem) and upper mainstem JDR. Statistical analyses were conducted in R (version 3.3.2; R Development Core Team 2016).

Six traits were analyzed throughout the invasion extent of rusty crayfish: carapace length, chela length, weight, trophic position, physiological condition, and sex ratio (the proportion of males at a site). Only data for crayfish caught by kick-seining, hand-netting, and snorkeling were included in the analysis, due to the known size and sex bias of trapping for large males (Larson & Olden 2016). To control for the strong relationship between body size, crayfish weight, and chela length due to allometric growth, residuals from carapace length-weight and carapace length-chela length non-linear regressions (hereafter ‘relative weight’ and ‘relative chela length’) were used as response variables in the models.

A subset of the variables measured at each site was selected as the main environmental predictors of crayfish traits: the estimated number of degree days from August 2015 to July 2016 (°C), macroinvertebrate AFDW (mg), and chlorophyll *a* concentration from benthic green algae and diatoms (μg Chl-a/cm^2). Degree days were computed based on water temperature estimated from a multiple regression model using satellite-measured daily land-surface temperature, calendar day, watershed area, and elevation as predictor variables (Chapter 1). *In situ* temperature measurements were not used in this analysis, as diel temperature variations were on the same order of magnitude as differences between upstream and downstream sites. Velocity and depth measurements were not included in the analysis either due to their high spatial variability at base flow in the JDR. Only the sites for which all variables had been measured were included in the analysis for a total of 19 sites, 15 sites in the mainstem JDR, 2 in the South Fork JDR, and 2 in the North Fork JDR.

In the South Fork and North Fork JDR, where tissue samples were taken from crayfish in only two sites, differences in traits among sites were tested using two-sampled t-tests (or Welch’s t-test in case of unequal variance and Mann-Whitney U test for non-normal data), and differences in sex ratio were assessed with Yate's Chi-square test. In the mainstem JDR, Generalized Additive Models (GAM) were developed to analyze the drivers of crayfish morphology, physiological condition, and trophic position. Models were fit in the *mgcv* package in R using thin plate regression splines and generalized cross validation criteria (GCV) for smoothing parameter estimation (Wood & Wood 2007). GAM models were built separately for two main categories of predictor variables. A first category of models was developed to account for the role of the range expansion process itself in driving phenotypic changes by using each site’s distance from the initial location of introduction of rusty crayfish as the predictor variable. The second model category was based on environmental variables that might influence the trait at hand. Additional models were also built using distance from introduction together with crayfish density and sex ratio as predictor variables, or combining multiple environmental variables. The significance and fit of candidate models were then compared among invasion fronts to determine whether consistent patterns arose.

2.5 Results

2.5.1 Crayfish distribution and habitat conditions

In total, 1266 crayfish were captured across the 19 sites analyzed in this study, of which 299 were sampled for morphological traits, 259 for physiological condition, and 254 for trophic position. Our survey and predictive model (Chapter 1) showed that rusty crayfish spread at an accelerating rate since its introduction and occupied at least 705 km of river across the JDR watershed in August 2016. By that summer, it had spread nearly 30 km upstream in the North Fork and South Fork JDR, and colonized the mainstem along a 250 km stretch downstream of its introduction point (Fig. 2.1). In contrast to its extensive spread downstream, the upstream spread of rusty crayfish in the mainstem had been temporarily halted at the time of the survey due to a low-head dam 12 km upstream of the putative site of crayfish introduction. Densities downstream of the dam were similar to those found at the core of their range. Therefore, the upstream mainstem invasion leading edge was not included in this analysis. In addition, the precise location of the downstream mainstem leading edge could not be determined due to limited access to the river; thus, the downstream-most surveyed site where rusty crayfish was found was treated as the downstream edge of their range in this study.

There was a consistent decrease in rusty crayfish densities among the sampled tributaries from their initial location of introduction to their invasion fronts. Rusty crayfish densities (measured as kick-seining catch per unit effort, Fig. 2.2) were highest in both the mainstem and South Fork JDR (> 30 crayfish/m^2) 40-75 km downstream of the initial site of rusty crayfish establishment, but rapidly dropped by an order of magnitude beyond 60 km in the South Fork JDR and beyond 80 km in the mainstem and North Fork JDR. Rusty crayfish and native signal crayfish were found in sympatry in only a few sites at the upstream invasion fronts of rusty crayfish, so interspecific competition was not considered as a significant mechanism influencing the traits investigated in this study.

Temperature and macroinvertebrate biomass followed mostly consistent longitudinal gradients from upstream to downstream in the mainstem, South Fork, and North Fork JDR, whereas benthic biomass of green algae and diatom were highly variable among tributaries both in terms of absolute value and longitudinal pattern (Fig. 2.1, Appendix 2.2). Degree days increased monotonically downstream while macroinvertebrate biomass decreased downstream. There was also considerable variability in macroinvertebrate biomass among adjacent sites and an upward shift past the confluence of the mainstem and the North Fork JDR, towards the low crayfish density areas of the mainstem. Green algae were sparse to absent in all tributaries (< 1.0 μg Chl-*a*/cm^2 in the North Fork and lower mainstem JDR), and though green algae biomass increased downstream in the lower and upper mainstem and the South Fork, it decreased downstream in the North Fork JDR. The biomass of diatoms, on the other hand, was highest in the South Fork and upper mainstem JDR (up to 4.5 μg Chl-*a*/cm^2) and in similar quantities as green algae in the lower mainstem and North Fork JDR, with inconsistent longitudinal gradients among tributaries.

2.5.2 Morphology

Rusty crayfish morphology showed weak yet consistent trends from core to leading edge populations in all tributaries (Table 2.1, Appendix 2.3). The relative chela length of rusty crayfish in leading-edge populations was significantly smaller than those behind the front in the North Fork JDR (Fig. 2.3A, Table 2.1; Mann-Whitney U, $p < 0.001$) and did not differ among sites in the South Fork JDR (Mann-Whitney U, $p=0.14$). In the mainstem JDR, there was no significant trend in chela length when considering distance from the location of initial rusty crayfish introduction alone (Appendix 2.3). However, the model that best predicted relative chela length along the mainstem included downstream distance and sex ratio as predictor variables, and explained 52% of the deviance in relative chela length — although sex ratio, by itself, was not consistently correlated with relative chela length across tributaries (Appendix 2.3, Appendix 2.4 Fig. 2.S2). Crayfish density was also positively associated with chela length in the mainstem (GAM, $p=0.03$, R^2-adjusted $=0.27$) and North Fork but not in the South Fork (Table 2.1), when taking into account all sites where more than 10 crayfish were captured. Whereas there was no consistent difference in mean carapace length between core and invasion front populations of the JDR or between male and female crayfish, there was a consistent decrease in carapace length variance in the direction of the invasion in both upstream and downstream dispersing populations (Table 2.1, Appendix 2.3), associated with a drop in maximum carapace length. Crayfish relative weight significantly decreased towards the invasion fronts of rusty crayfish in the North Fork (t-test, $p < 0.001$) and mainstem (GAM, $p=0.003$, R^2-adjusted $=0.49$), and non-significantly decreased in the South Fork JDR (Appendix 2.4 Fig. 2.S3C), along with crayfish density (Appendix 2.3). Lastly, there was no significant trend in the proportion of males towards the fronts of the invasion despite a slight increase in sex ratio in both upstream and downstream leading edges, when considering all sites where more than 10 crayfish were captured (Appendix 2.4 Fig.2.S4).

Environmental conditions were not strong predictors of crayfish morphology throughout their invasion gradient. Degree days did not correlate consistently across invasion fronts with any morphological trait (Table 2.1, Appendix 2.3). For instance, while decreasing upstream temperatures were associated with a decrease in relative weight in the South Fork and North Fork JDR, relative weight and temperature were negatively correlated in the mainstem. Similarly inconsistent patterns were observed between temperature and relative chela length and between temperature and carapace length. Differences in biomass (AFDW) of macroinvertebrates among sites were negatively correlated with mean crayfish relative weight across tributaries (Table 2.1, Appendix 2.3) but were not significantly associated with shifts in carapace length or relative chela length. Finally, while green algae biomass was not consistently correlated with any morphological trait, diatom biomass was positively correlated with relative weight throughout the JDR (Table 2.1, Appendix 2.3).

2.5.3 Trophic position

The trophic position of rusty crayfish was consistently lower for individuals at invasion fronts than in populations closer to the core, despite wide variations among sites throughout the watershed (Table 2.1, Fig. 2.3B). In the mainstem, rusty crayfish diet first increased downstream from that typical of a secondary consumer or omnivore (trophic position of ~3) to that of a top carnivore (trophic position of ~4) and then decreased towards the front of the invasion down to that of a primary consumer (trophic position of ~2; ; GAM, $p=0.05$, R^2-adjusted $=0.44$; Fig. 2.3B). There was an equivalent drop in crayfish position in the North Fork JDR upstream leading edge (t-test, $p < 0.001$) but no equivalent decrease in the South Fork JDR leading edge. Although crayfish density alone did not significantly correlate with trophic position, it was negatively correlated with trophic position when included in a model together with distance from the introduction point (GAM, crayfish density: $p=0.08$). There was no difference in trophic position among male and female crayfish, and although trophic position was weakly correlated with carapace length within sites (carapace length fixed effect 95% CI= $8.0 \cdot 10^{-3}$ to $1.9 \cdot 10^{-2}$ TP/mm in linear mixed effect model with site as random effect), there was no significant correlation between trophic position and mean carapace length across sites along the mainstem.

Temperature, collinear with downstream distance from the invasion source, was negatively correlated with trophic position in the mainstem (GAM, $p=0.03$, R^2-adjusted=0.47) but decreased along with trophic position towards the invasion front in the North Fork and South Fork JDR (Table 2.1, Appendix 2.2). Neither macroinvertebrate biomass nor green algae significantly covaried with trophic position in a consistent way across tributaries, but diatom biomass exhibited a positive relationship with trophic position in all fronts (Table 2.1, Appendix 2.2; GAM, $p=0.06$, R^2-adjusted =0.33).

2.5.4 Growth and condition

There was a consistent positive trend in rusty crayfish physiological condition towards invasion fronts (Fig. 2.3C, Table 2.1). Strongest towards the upstream leading edges despite decreasing temperature (t-test, South Fork: $p < 0.01$; North Fork: $p < 0.001$), the increase in physiological condition was only marginal in the mainstem [GAM, $p=0.19$, R^2-adjusted=0.06, mean slope=$6.3\cdot10^{-3}$ (95% CI=$-2.7\cdot10^{-3}$ to $1.5\cdot10^{-2}$) (RNA/DNA)/km]. Neither environmental variables (abiotic and biotic) nor crayfish density were significantly associated with RNA/DNA ratio across tributaries (Table 2.1).

2.6 Discussion

This study revealed significant trends in morphological traits, physiological condition, and trophic position of rusty crayfish from their location of initial introduction to the leading invasion edge in the JDR, their only known population in western North America. Even though these trends were not consistently significant across tributaries, they suggest that rusty crayfish individuals at the vanguard of the invasion exhibited less competitive morphology (decreased relative weight and chela length) and used energetic pathways lower on the food chain, yet were in better physiological condition than individuals located closer to the invasion core. We contend that these phenotypic shifts observed in rusty crayfish are likely due to the range expansion process itself rather than plastic responses to novel environmental conditions at their range boundaries, and that these trends may grow stronger as the invasion continues to unfold.

The evolutionary potential of dispersal ability at the range boundaries of expanding populations has now been repeatedly demonstrated through empirical (Chuang & Peterson 2016), experimental (Ochocki & Miller 2017; Weiss-Lehman, Hufbauer & Melbourne 2017), and theoretical research (Burton, Phillips & Travis 2010). However, inference regarding whether and which traits are undergoing selection is often influenced by uncontrolled variations in environmental conditions among sampled sites. For instance, several studies have examined changes in growth rate, body size, body condition, and gut fullness in freshwater invaders; yet, many have concluded that the observed patterns are likely due to unknown differences in resource availability between front and core populations (Raby, Gutowsky & Fox 2010; Lopez, Jungman & Rehage 2012; Brandner *et al.* 2013; Pârvulescu *et al.* 2015). In this study, our survey was designed to encompass both upstream and downstream invasion fronts such that environmental conditions did not consistently covary with rusty crayfish density or the distance from their introduction point. This allowed us to partially disentangle the effect of the range expansion process from the influence of environmental conditions.

The observed decrease in crayfish relative chela length and weight towards the front of the invasion can be attributed to three main reasons – though further research is needed to confirm the respective contribution, if any, of each of these mechanisms. First, the range expansion of rusty crayfish might be driven by the exclusion of sub-dominant individuals from high density population centers, therefore leading to the widespread presence of competitively inferior crayfish at the invasion leading edge (Hudina, Hock & Zganec 2014). In other words, individual crayfish with less competitive phenotypes may have been pushed out and systematically interbred at the invasion front, resulting in the accumulation of light, small-clawed individuals at the leading edge. Signal crayfish at the boundary of their invasion range display lower levels of aggression and often lose agonistic interactions to individuals from the invasion core, despite being in better physical condition (Hudina, Zganec & Hock 2015). This process was similarly hypothesized to explain decreasing body size of invasion front round goby in the Trent-Severn waterway (Brownscombe & Fox 2012; Masson, Brownscombe & Fox 2016). However, a significant increase in relative claw size away from source population was also observed in invasive signal crayfish males of the Mura River in Croatia (Hudina *et al.* 2012). Second, larger chelae and a stouter body shape might be associated with slower crayfish movement and thus be selected against through associative mating of the fastest dispersers clustered at the invasion leading edge. Third, low rusty crayfish conspecific densities at invasion front sites may relax the constraints imposed by competition in high density areas and thus reduce the requirement for investment in traits associated with competition. Shelter and food limitations are the main drivers of agonistic interactions in crayfish (Capelli & Hamilton 1984; Bergman & Moore 2003). When these resources are plentiful in pioneer populations, behavioral phenotypes associated with large claws and weight as part of an aggression syndrome (Hudina *et al.* 2012) could therefore lead to unnecessary energy expenses and reduced foraging, leading to reduced fitness (Sih *et al.* 2012). A shift in selective pressure might thus have led to a change in rusty crayfish life history at the edge of the invasion, involving the reallocation of energy from allometric growth to reproduction and dispersal traits not measured here (e.g. walking leg length; Burton, Phillips & Travis 2010; Phillips, Brown & Shine 2010b). While some selection for competitive ability in rusty crayfish could be expected as it interacts with signal crayfish in frontier areas, the lack of sympatry of these two species in our survey, the low densities of signal crayfish even in uninvaded areas, and the greater rates of somatic growth of rusty crayfish young-of-the-year documented by Sorenson (2012) all suggest a minor impact of inter-specific competition on selection in pioneer populations. Without knowledge of the heritability of the crayfish traits examined in this study, inference regarding the specific mechanisms in operation is still limited.

We conjecture that a shift in the selection regime experienced by rusty crayfish from core to leading edge areas is the most likely explanation for the trends in relative chela length and weight observed in this study. The accelerating rate of spread of rusty crayfish in the JDR, the low population densities observed within several kilometers of the upstream and downstream invasion fronts, and high physiological fitness in invasion front populations suggest that a pushed invasion excluding subdominant crayfish from higher density areas is unlikely to explain the observed trait differences. Moreover, there is little to no evidence that chela size or relative body weight are associated with dispersal speed. Lastly, the difference in relative weight and chela length between invasion core and front populations could also be driven by natural selection in high density core populations. Historical data from Sorenson (2012), combined with this study, show a significant increase in relative weight from 2010 to 2016 at the presumed site of rusty crayfish introduction but no difference in chela length (Appendix 2.5). The changes in traits observed here are thus likely the results of a combination of drivers including reduced fitness of competitive phenotypes at low crayfish density, selection for high relative weight in the population core, and trade-offs between these competitive attributes and other traits associated with higher dispersal ability. These trends are also consistent with the narrower range in size distribution and lower maximum carapace length observed in rusty crayfish pioneer populations, given that differences in carapace length strongly predict social dominance in crayfish (Rabeni 1985).

The observed increase in rusty crayfish anabolic activity, measured as RNA/DNA, in leading edge populations of the JDR indicates that the range expansion process has led to greater somatic growth and/or reproductive potential in pioneer individuals. This pattern matches several documented increases in body condition, growth, and reproductive potential in similar stream invasions. For example, invasion front signal crayfish in Croatia were in better condition and energetic status, and exhibited higher potential for energetic investment into reproduction than conspecifics in the invasion core (Rebrina *et al.* 2015). Similarly, invasive female spiny-cheek crayfish had more ovarian eggs at the front of their invasion in the Lower Danube than in core areas, and this frontier phenotype was replaced at that then-leading-edge site in subsequent invasion stages (Pârvulescu *et al.* 2015). Nonetheless, whether the observed increase in rusty crayfish physiological condition towards the invasion vanguard represents greater somatic growth rates or gamete production remains unresolved and warrants further investigation. Indeed, simultaneous increases in condition, growth, and reproductive potential are not universal because selection for dispersal ability during range expansion may lead to unexpected trade-offs. For instance, tadpoles and juveniles in invasion-front populations of cane toads in tropical Australia grow up to 31% faster than those from longer established populations (Phillips 2009), and adults demonstrate higher feeding rates, larger fat stores, and better condition than conspecifics in later invasion stages (Brown, Kelehear & Shine 2013). However, lower reproductive rates have also been documented in invasion front populations (Hudson *et al.* 2015)

It might seem that higher crayfish growth rates towards the invasion fronts contradict the observed patterns of reduced weight and chela length observed in pioneer crayfish. However, selection for a faster lifestyle could decrease age at maturity and shorten the lifespan of these invasion front crayfish while selecting against allometric growth of competitive morphology. Both crayfish growth rate and fecundity are density dependent (Momot & Gowing 1977; Guan & Wiles 1999). Therefore, it is possible that high growth and fecundity phenotypes have arisen from natural selection in the ~15 generations since their introduction as part of a dispersal syndrome, associating rapid development and high fecundity with dispersive traits (Ronce & Clobert 2012; Stevens *et al.* 2013). In conditions of low conspecific densities at the expanding edge of an invasive population, the genotype of those crayfish that grow the fastest to maturity and produce the most offspring not only is most likely to be passed on and selected for in subsequent generations, but is also more likely to reach the invasion front in next generation if the parents are fast dispersers (Burton, Phillips & Travis 2010).

Rusty crayfish at the leading edges of their distributional limits appear to be feeding lower in the food web when compared to conspecifics located behind the invasion front. This pattern stands in contradiction with most previous studies of stream invasions. Round gobies at the edge of their expanding range consume more of their favored prey type than in central populations (Raby, Gutowsky & Fox 2010) and have higher $\delta^{15}N$ signatures than the previous year front (Brandner *et al.* 2013). Invasion front bloody red mysid shrimp (*Hemimysis anomala*) were not more selective in their prey consumption, but showed greater ability to locate and capture zooplankton prey than those shrimp in core populations (Iacarella, Dick & Ricciardi 2015). It is unlikely that cannibalism, a widespread phenomenon in crayfish populations (Guan & Wiles 1998), led to the observed increase in trophic position in areas with high densities of crayfish, as conspecific density was not correlated to trophic position in the JDR (Table 2.1, Appendix 2.3).

Rusty crayfish had a lower trophic position even when macroinvertebrate prey availability increased towards the invasion front in the North Fork JDR. This pattern contrasted with past evidence of a positive relationship between macroinvertebrate availability and crayfish trophic position (Olsson et al. 2008) and greater assimilation efficiencies of invertebrates than other food items by crayfish (Whitledge & Rabeni 1997). However, the lack of significant decrease in trophic position towards the invasion front in the South Fork JDR could be due to a counter-effect from increasing macroinvertebrate biomass upstream. Consumption of macroinvertebrates has been linked to increases in signal crayfish weight gain by a factor of 2 to 3 when compared to leaf- or wood-based diets (Bondar *et al.* 2005) and rusty crayfish gained more than five times more weight and achieved greater metabolic rates on an invertebrate diet than on detritus or periphyton diets (Hill, Sinars & Lodge 1993; McFeeters *et al.* 2011). Nevertheless, in these same studies, juvenile signal crayfish in a natural setting disproportionately consumed food types that were the opposite of those shown to be of most nutritional value to them (Bondar *et al.* 2005) and rusty crayfish mortality was higher on a diet based on invertebrate than one on periphyton or detritus (Hill, Sinars & Lodge 1993). This suggests that high growth might be associated with greater physiological stress, due to more frequent molting, and higher foraging costs in natural settings. The energy intake and long term fitness of crayfish associated with periphyton and detritus consumption may thus be higher than with macroinvertebrate diets despite their lower digestion efficiency of these resources.

Trade-offs can also arise between increased dispersal rates at range margins and the functional response of the non-native consumer due to the high cost of dispersal (Fronhofer &

Altermatt 2015). Given their current rate of spread (~20 km/year downstream from 2010 to 2016) in the JDR and a window of activity of 8-9 months (based on water temperatures in the mainstem), rusty crayfish would have to achieve a net downstream spread rate of 80 m per day on average, without considering time dedicated to mating and juvenile parental care. We speculate that this pace could restrict the amount of time available for actively preying on invertebrates, and could select for those crayfish best able to efficiently feed and grow on abundant and accessible basal resources. It has also been hypothesized that those species that can sustain growth and reproduction on low-quality resources should be best able to become established into a stream community (Gido & Franssen 2007). In crayfish, a broader trophic niche that expanded towards lower trophic levels might have afforded competitive advantages to the introduced signal crayfish over the native noble crayfish *Astacus astacus* in Swedish streams (Olsson *et al.* 2009). Therefore, our findings that invasion front rusty crayfish act mostly as primary consumers while achieving greater physiological condition might reflect an increase in feeding efficiency on basal resources as a byproduct of greater dispersal ability developed through their range expansion in the JDR.

Our simultaneous study of the range expansion of this invasive species in multiple tributaries of the JDR highlighted the potential that river systems hold for studying range expansion. This design allowed us to leverage the consistency of observed trends across invasion fronts to infer which mechanisms were in operation. Nevertheless, we also expect greater evolutionary stochasticity to occur in river systems than terrestrial systems in the specific traits undergoing selection and in the magnitude of phenotypic change. Repeated founder events during the range expansion process can lead to stochastic trait selection and even gene surfing by deleterious alleles similar to genetic drift (Amundsen *et al.* 2012; Phillips 2015; Weiss-Lehman, Hufbauer & Melbourne 2017). Because these stochastic effects are exaggerated in one-dimensional space, there is heightened potential for unpredictable outcomes in unidirectional river systems (Phillips 2015).

Improved understanding of the distribution and eco-evolutionary drivers of rusty crayfish phenotypes throughout the JDR represents a crucial first step towards developing spatially explicit strategies to control this invasion. If the documented phenotypic shifts from core to invasion front populations are indeed contributing to the accelerating rate of spread of rusty crayfish, then targeting those individuals at the invasion leading edge for removal (e.g. by trapping) might constrain the accumulation of dispersive phenotypes in these areas. Accounting for these phenotypic shifts in mechanistic models of invasive spread (e.g. Chapter 1) could also provide us with a virtual laboratory to test the effectiveness of alternative control strategies in containing the spread of riverine invaders like rusty crayfish.

Our results suggest that low conspecific densities and spatial sorting in leading edge populations led to a shift in the phenotype of rusty crayfish towards higher intrinsic growth and/or reproduction, lower competitive ability and greater foraging efficiency on basal resources, as they spread upstream and downstream in the JDR. Our study design enabled us to link morphological and functional traits to better grasp the consequences of this range expansion on invaded ecosystems. We expect that the diminished competitive ability observed in the vanguard of this rusty crayfish invasion might lead to reduced fitness of the invasion front phenotypes once densities in newly colonized areas limit the availability of shelter and intensify competition for mates. The long-term evolutionary implications of these phenotypic shifts might thus be limited (Perkins, Boettiger & Phillips 2016). However, the trophic shift observed in invasion front populations could also allow rusty crayfish to reach higher densities in these areas, as they might exploit resources more broadly and efficiently under competitive conditions. The evolutionary forces at play in this invasion have likely interacted with longitudinal gradients in environmental conditions in ways analogous to those experienced by species during their migration towards cooler areas under climate change. Integrating the study of morphological and functional traits with spatial variation in environmental conditions thus provides a robust way to assess whether contemporary evolution is altering the phenotype and ecosystem impacts of species as their range expands through the landscape.

2.7 Author's contributions

M. L. M. and J. D. O. conceived and designed the study, obtained funding, collected data, interpreted the data, and prepared the manuscript. M. L. M. performed the laboratory work and analyzed data.

2.8 Acknowledgements

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2.10 Figures and tables

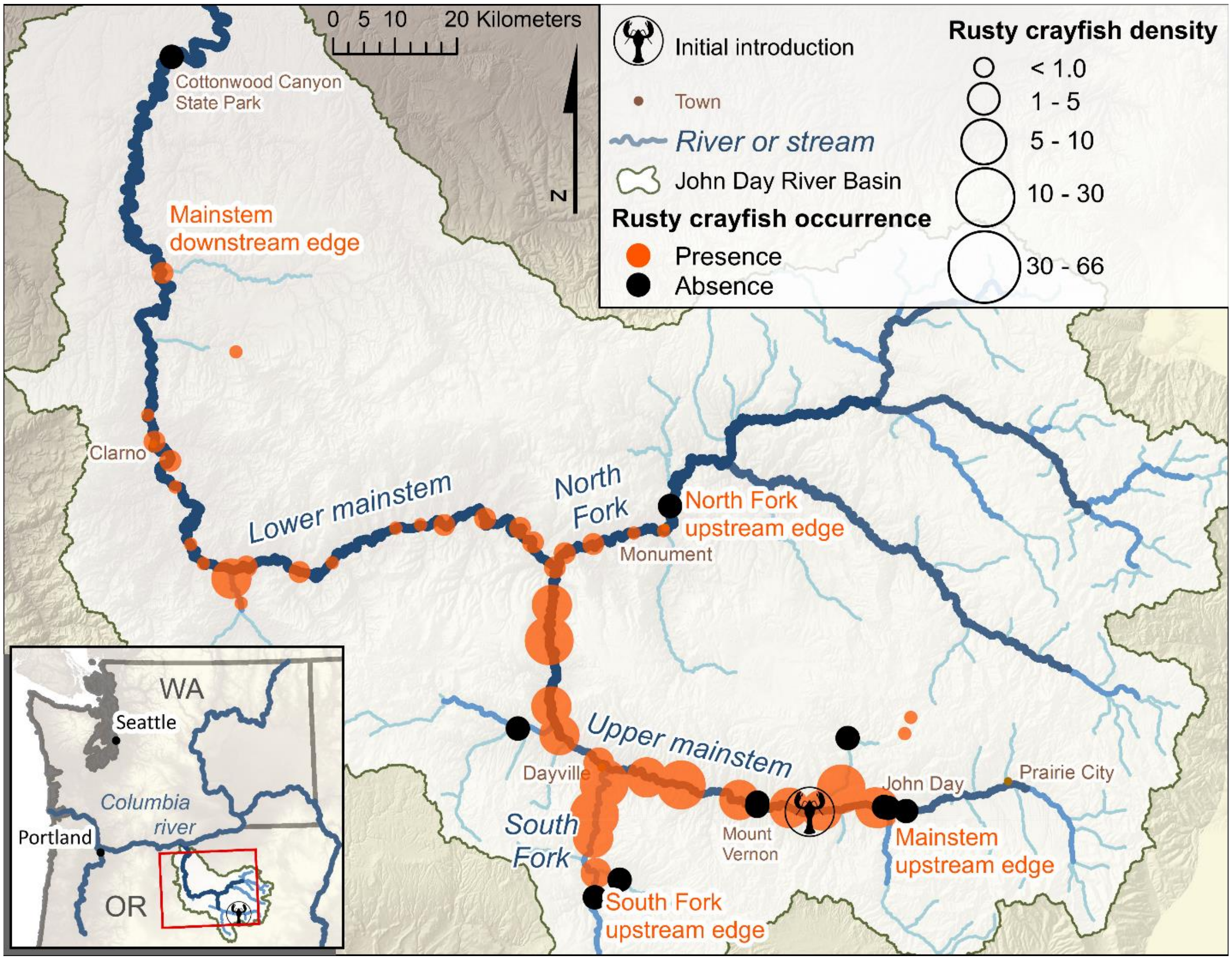

Figure 2.1. Regional map of the John Day River basin (JDR, inset) and relative densities of rusty crayfish along the mainstem, North Fork, and South Fork of the JDR in 2016 (circle size), with the presumed point of initial introduction (crayfish symbol).

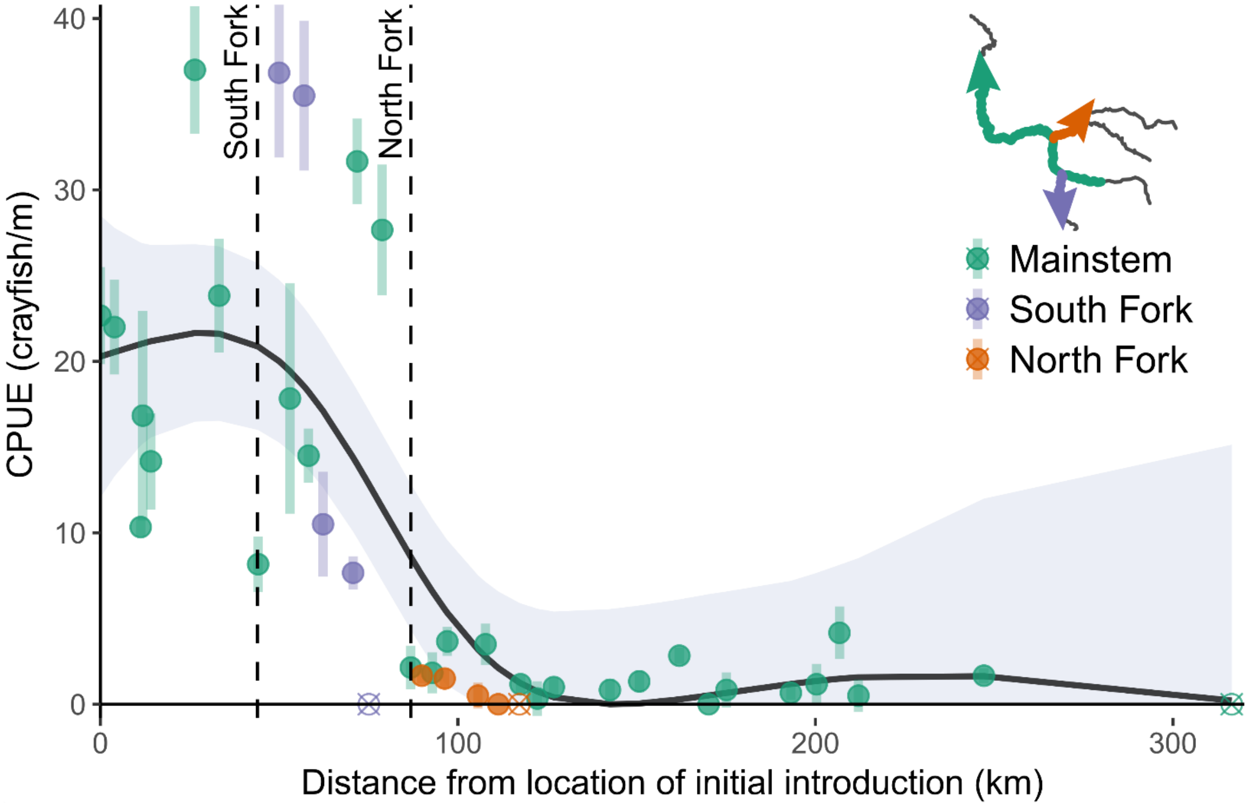

Figure 2.2. Declining densities of rusty crayfish (y-axis) with increasing distance from their location of initial introduction (x-axis) in the mainstem (green), North Fork (orange) and South Fork (purple) John Day River. Crayfish densities are expressed as the mean (points) and 95% confidence interval (bars) of the catch per unit effort (CPUE) from six standardized 1-m^2 kick-seine samples at each site. The smooth solid line and shaded region represent the predicted mean CPUE and 95% Bayesian credible interval, respectively, from a Generalized Additive Model (GAM). Vertical dashed lines represent confluences of the South Fork (at 44km) and North Fork (at 87 km) with the mainstem JDR.

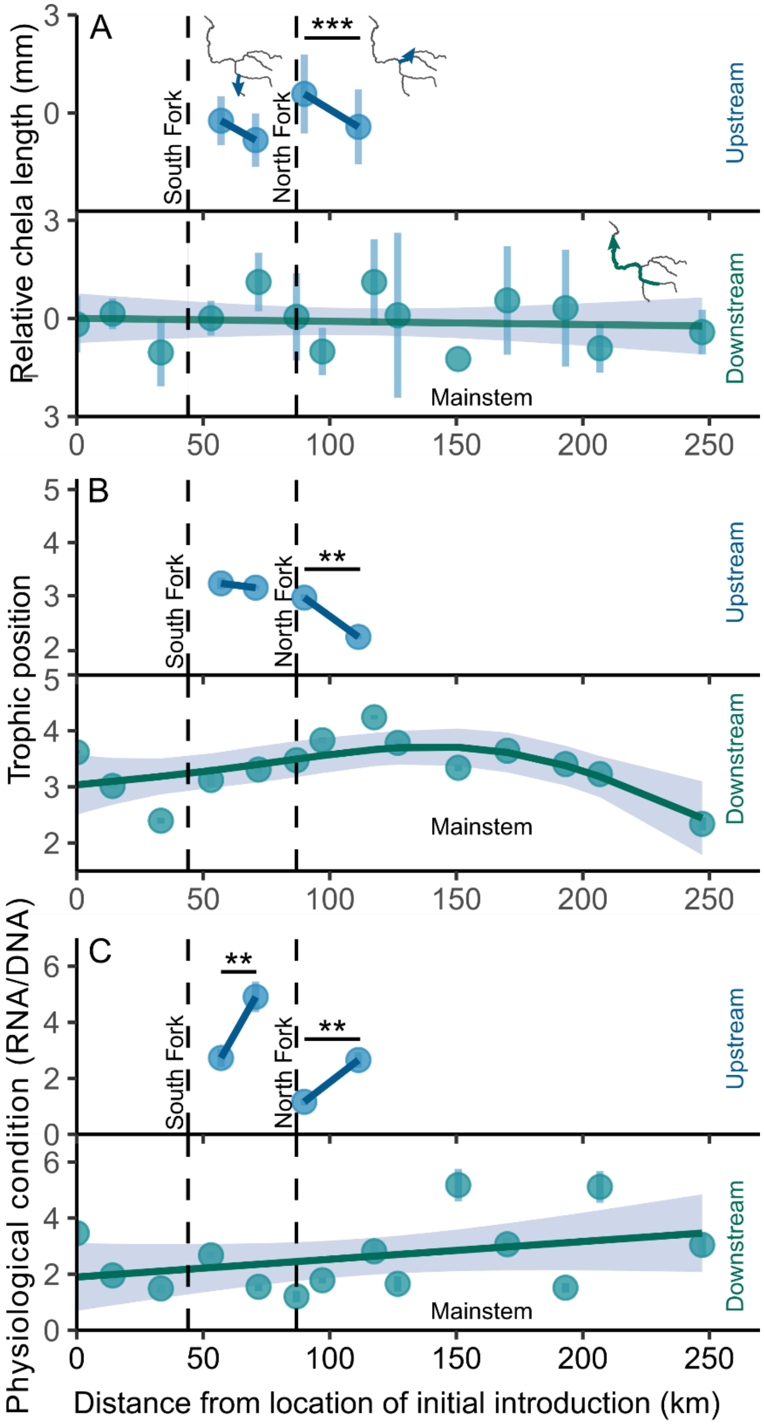

Figure 2.3. Changes in mean crayfish relative chela length (A), trophic position (B), and physiological condition (C; RNA/DNA ratio expressing potential for somatic growth and gamete production) along the invasion gradient of rusty crayfish in the JDR watershed. Consistent changes upstream (top graphs) and downstream (bottom graphs) suggest that differences in rusty crayfish traits stem from the range expansion itself rather than from longitudinal gradients in environmental conditions. The smooth solid line and shaded region represent the predicted mean

trait value and 95% Bayesian credible interval, respectively, from Generalized Additive Models (GAM) and asterisks denote significant differences in mean ('***': $p \leq 0.001$, '**': $p \leq 0.01$). Vertical dashed lines represent the confluences of the South Fork (at 44km) and North Fork (at 87 km) with the mainstem JDR.

Table 2.1. Summary statistics of tests for phenotypic differences between rusty crayfish individuals near invasion fronts and those closer to the core in the mainstem, North Fork, and South Fork John Day River, with respect to relative chela length, trophic position, physiological condition (RNA/DNA), carapace length (CL) mean and variance, relative weight, and sex ratio. The direction of the relationships between a predictor variable and a response variable are denoted by '+' and '–' whereas '/' indicates a non-monotonic pattern in the relationship (e.g. +/- denote an increase followed by a decrease in trait value along the range of the independent variable). Bolded signs represent significant trends; significance is reported here for the South Fork and North Fork JDR ('***'≤ 0.001, '**'≤ 0.01, '*'≤ 0.05 , '·'≤ 0.1), see Appendix 2.3 for a summary of GAM outputs. Significance and a consistent direction in the relationship between candidate predictors and traits among tributaries suggested the primacy of that predictor in driving observed differences in traits. Differences in predictor variables among sites in the South Fork and North Fork JDR were not formally assessed as measures of variance were not available for all variables. Relationship with 'N.A' were not tested because of a lack of hypothesized mechanisms relating the variables.

Response variable	Edge	Test | p-value	Predictor variable					
			Distance from introduction	Crayfish density	Degree days	Macroinv. AFDW	Green algae	Diatom
Relative chela length	Downstream: Mainstem	GAM (n=14)	-	+	-	-	-	-
	Upstream: South Fork	Mann-Whitney U | 0.14	-	0	+	-	0	+
	Upstream: North Fork	Mann-Whitney U | ***	-	+	+	-	-	+
Trophic position	Downstream: Mainstem	GAM (n=14)	+/-	-	+/-	+	0	-/+
	Upstream: South Fork	Mann-Whitney U | 0.9	-	+	+	-	0	+
	Upstream: North Fork	t-test | ***	-	+	+	-	-	+
RNA/DNA	Downstream: Mainstem	GAM (n=14)	+	-	+	0	+	0
	Upstream: South Fork	Mann-Whitney U | **	+	-	-	+	0	-
	Upstream: North Fork	t-test | ***	+	-	-	+	+	-
CL	Downstream: Mainstem	GAM (n=14)	+/-	-/+	+	+	+	-
	Upstream: South Fork	Mann-Whitney U | 0.2	+	-	-	+	0	-
	Upstream: North Fork	Mann-Whitney U | 0.7	-	+	+	-	-	+
CL variance	Downstream: Mainstem	GAM (n=14)	-	+	N.A.	N.A.	N.A.	N.A.
	Upstream: South Fork	Levene's test | ·	-	+	N.A.	N.A.	N.A.	N.A.
	Upstream: North Fork	Levene's test | **	-	+	N.A.	N.A.	N.A.	N.A.
Relative weight	Downstream: Mainstem	GAM (n=14)	-	+	-	-	-	+
	Upstream: South Fork	Mann-Whitney U | 0.2	-	+	+	-	0	+
	Upstream: North Fork	t-test | ***	-	+	+	-	-	+
Sex ratio	Downstream: Mainstem	GAM (n=21)	+	N.A.	N.A.	N.A.	N.A.	N.A.
	Upstream: South Fork	Yate's Chi-square | 0.9	+	N.A.	N.A.	N.A.	N.A.	N.A.
	Upstream: North Fork	Yate's Chi-square | 0.9	+	N.A.	N.A.	N.A.	N.A.	N.A.

2.11 Appendices

2.11.1 Appendix 2.1: reagents specifications

Table 2.S1. Name and reference of reagents used in RNA and DNA quantification.

Reagent name	**Reference**
RNA*later*®	Sigma-Aldrich #R0901
N-Lauroylsarcosine sodium salt	Sigma-Aldrich #L9150
Quant-iT™ RiboGreen® RNA Assay Kit	Molecular Probes, #R11490
DNase-free RNase	ThermoFisher #EN0531
RNase-free DNase	ThermoFisher #89836
Ribosomal RNA standard, 16S and 23S rRNA from *E. coli*	Molecular Probes, #R11490 Component C
Calf Thymus DNA standard	Sigma, #D3664

2.11.2 Appendix 2.2: longitudinal gradients in environmental conditions in the John Day River

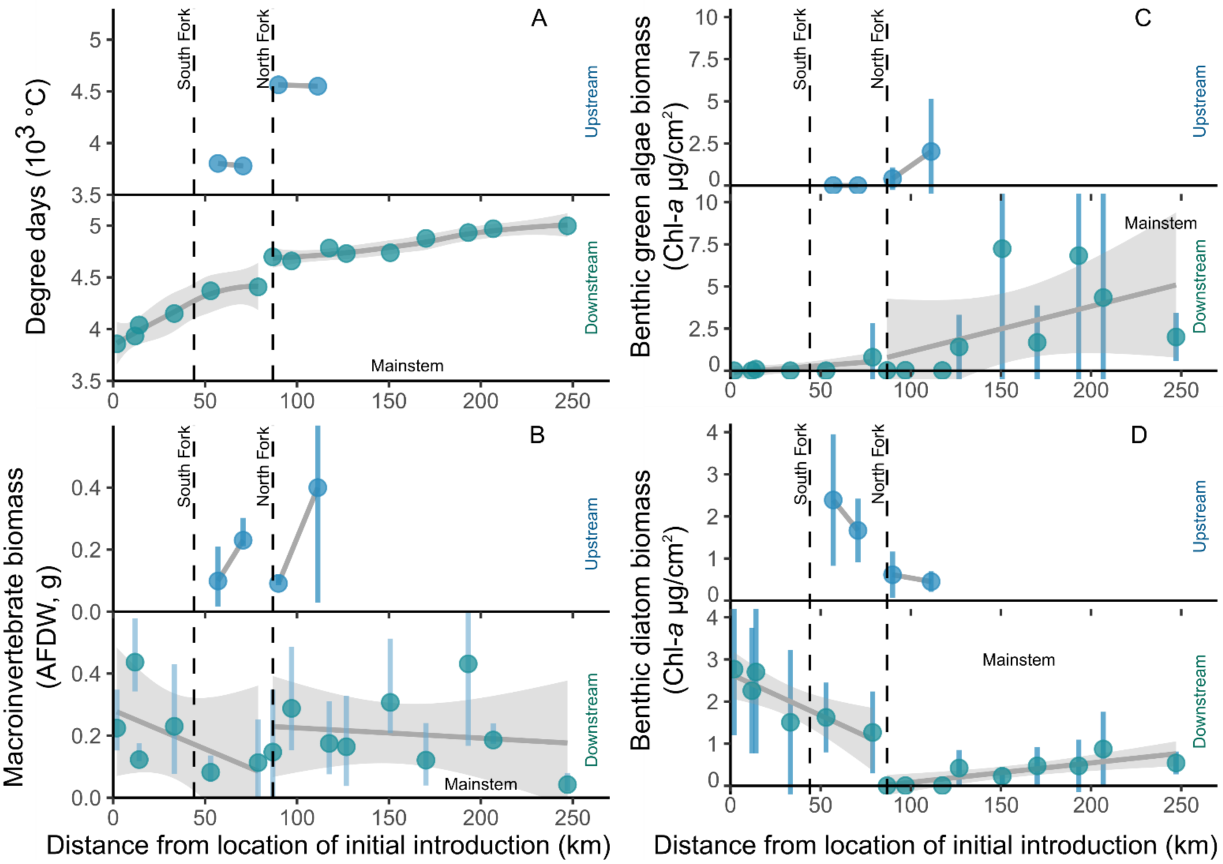

Figure 2.S1. Longitudinal variations in degree days (A, y-axis), macroinvertebrate biomass (B, y-axis, Ash Free Dry Weight), benthic green algae (C, y-axis) and diatom biomass (D, y-axis) with respect to the distance from the initial location of introduction of rusty crayfish downstream in the mainstem (lower panels) and upstream in the South Fork and North Fork (upper panels) JDR. Points represent mean values and error bars represent 95% confidence intervals. The grey lines and shaded areas represent predictions from a loess fit (A) and linear regressions (B-D). Vertical dashed lines represent the confluences of the South Fork (at 44 km) and North Fork (at 87 km) with the mainstem JDR. Benthic algal biomass is expressed as the concentration of Chlorophyll-*a* in µg.cm^{-2} on 10 cobbles randomly selected at regular intervals in a transect perpendicular to flow direction.

2.11.3 Appendix 2.3: summary table of General Additive Models (GAM) outputs

Table 2.S2. Summary table of outputs from General Additive Models (GAM) of rusty crayfish traits throughout their invasion range in the mainstem of the John Day River. Candidate predictor variables included the distance from the initial location of rusty crayfish introduction (distance from introduction, km), the proportion of males at each site (sex ratio, %), the density of crayfish measured as mean kick-seining catch per unit effort (crayfish/m^2), the estimated number of degree days at the site from July 2015 to August 2016 (°C), the biomass of macroinvertebrates measured as mean ash-free-dry-weight from D-frame netting (g), and the mean benthic biomass of green algae and diatoms measured with a Benthotorch (Chl-*a* μg.cm^{-2}). The | sign separates model parameters for different predictor variables, + and – in the *sign* column denote the direction of the relationship between a predictor variable and the response variable while / indicates a non-monotonic pattern in the relationship towards the downstream front. GAMs were developed in the *mgcv* package in R using thin plate penalized regression and were evaluated using the Akaike Information Criterion (AIC), the p-value of the coefficients, the adjusted R^2, the effective number of degrees of freedoms of the smoothing function for each predictor variable (smooth edf), the basis dimension (k in *mgcv* package, determining the maximum degrees of freedom, to avoid overfitting), and estimated concurvity when multiple independent variables were included in the model (a generalized measure of multicollinearity computing nonlinear dependencies among the predictor variables). All models were compared to an intercept model, sorted by row from low to high AIC, and were based on data from 14 sites.

Response	Predictor	Sign	AIC	p-value	R^2 (adj)	smooth edf	Basis dim.	Concurvity (est.)
Relative chela length	Distance from intro. + sex ratio (n=14)	- | +	29	0.06|0.006	0.43	1.0|1.0	3|2	0.43|0.33
	Sex ratio (n=14)	+	31	0.03	0.27	1	9	-
	Crayfish density (n=21)	+	45	0.03	0.27	1.83	2	-
	Sex ratio (n=21)	+	48	0.07	0.12	1	9	-
	Distance from intro. + sex ratio (n=21)	- | +	48	0.14|0.03	0.18	1.0|1.0	9|9	0.41|0.33
	Intercept (n=14)	*NA*	*35*	-	-	-	-	-
	Intercept (n=21)	*NA*	*50*	-	-	-	-	-
	Green algae	-	35	0.30	0.01	1.0	9	-
	Crayfish density (n=14)	+	36	0.58	0.01	1.47	3	-
	Macroinvertebrate AFDW	-	36	0.29	0.02	1.0	2	-
	Distance from introduction	-	37	0.75	-0.07	1.0	9	-
	Invasion year	-	37	0.86	-0.08	1.0	9	-
	Degree days	-	37	0.91	-0.08	1.0	9	-
	Diatom	-	37	0.87	-0.08	1.0	9	-
	Distance + crayfish density	-|-/+	37	0.49|0.62	-0.02	1.0|1.6	9|3	0.83|0.60
Trophic position	Invasion year	+/-	17	0.03	0.49	2.7	3	-
	Distance from introduction	+/-	18	0.05	0.44	2.6	3	-
	Degree days	+/-	18	0.03	0.47	2.9	3	-
	Distance from intro. + crayfish density	-|-	19	0.09|0.08	0.43	1.38|1.61	2|2	0.62|0.59
	Diatom	-/+	20	0.06	0.33	1.8	2	-
	Degree days + diatom	0|-/+	20	0.68|0.04	0.33	0|1.5	3|2	0.73|0.87
	AFDW + diatom	+/-|-/+	20	0.26|0.04	0.34	1.0|1.5	2|2	0.07|0.05
	Degree days + AFDW	+|+/-	23	0.23|0.15	0.14	0.6|0.6	3|2	0.10|0.13
	Intercept	*NA*	*24*	-	-	-	-	-
	Macroinvertebrate AFDW	+	24	0.43	0.12	2.1	9	-
	Degree days + crayfish density	-|-	24	0.19|0.22	0.14	1.0|1.7	2|3	0.80|0.70
	Crayfish density	-	25	0.25	0.04	1.0	9	-
	Carapace length	+	25	0.23	0.04	1.0	2	-
	Green algae	0	26	0.86	-0.08	1.0	9	-
RNA/DNA	AFDW(k=1) +Green algae	0|+	47	0.17|0.03	0.26	1.0|1.0	0|1	0.90|0.35
	Green algae	+	48	0.07	0.20	1.0	6	-
	Intercept	*NA*	*50*	-	-	-	-	-
	Distance from introduction	+	50	0.19	0.06	1.0	9	-
	Invasion year	+	50	0.20	0.06	1.0	9	-
	Degree days + Green algae	-|+	50	0.85|0.13	0.13	1.0|1.1	9|2	0.67|0.43
	Crayfish density	-	51	0.43	-0.03	1.0	9	-
	Degree days	+	51	0.51	0.03	1.4	9	-
	Macroinvertebrate AFDW	0	52	0.95	-0.08	1.0	9	-
	Diatom	0	52	0.91	-0.08	1.0	9	-
	Distance from intro. + crayfish density	+|0	52	0.29|0.85	0.00	1.0|1.18	9|4	0.61|0.81
Carapace length	Distance from intro. + crayfish density	-|-/+	71	0.07|0.14	0.40	1.0|1.9	3|3	0.59|0.62
	Distance from introduction	+/-	73	0.14	0.31	2.5	3	-
	Invasion year	+/-	73	0.12	0.32	2.6	3	-

	Macroinvertebrate AFDW	+	75	0.14	0.10	1.0	9	-
	Diatom	-	75	0.15	0.10	1.0	9	-
	AFDW + Diatom	+|0	75	0.36|0.17	0.23	2.2|1.0	6|6	0.44|0.51
	Intercept	*NA*	*76*	-	-	-	-	-
	Crayfish density	-/+	76	0.46	0.09	1.3	9	-
	Degree days	+	77	0.51	-0.04	1.0	7	-
	Green algae	+	77	0.39	-0.02	1.0	2	-
	AFDW + Green algae	+|0	77	0.26|0.99	0.02	1.0|1.0	4|4	0.60|0.54
Relative weight	Crayfish density	+	-35	0.003	0.49	1.0	9	-
	Degree days + AFDW	-|-	-34	0.01|0.04	0.50	1.0|1.0	9|4	0.16|0.52
	Distance from intro. + crayfish density	+|-	-33	0.04|0.75	0.45	1.0|1.0	6|6	0.64|0.67
	Degree days + AFDW + Green algae	-|-|+	-33	0.02|0.07|0.54	0.47	1.0|1.0|1.0	9|2|2	0.46|0.87|0.85
	Degree days	-	-31	0.02	0.34	1.0	9	-
	Degree days + Green algae	-|-	-30	0.07|0.61	0.34	1.0|1.3	9|2	0.43|0.67
	Distance from introduction	-	-29	0.03	0.26	1.0	9	-
	Invasion year	-	-29	0.04	0.25	1.0	9	-
	Diatom	+	-29	0.05	0.23	1.0	8	-
	Degree days + Diatom	-|+	-29	0.20|0.85	0.28	1.0|1.0	9|2	0.66|0.98
	Green algae + Diatom	-|+	-29	0.18|0.11	0.29	1.0|1.0	2|2	0.13|0.12
	Macroinvertebrate AFDW	-	-28	0.07	0.19	1.0	9	-
	Green algae	-	-28	0.08	0.17	1.0	9	-
	Intercept	*NA*	*-26*	-	-	-	-	-
	Sex ratio	-	-25	0.45	-0.03	1.0	9	-
Sex ratio	*Intercept*	*NA*	*103*	-	-	-	-	-
	Distance from introduction (n=21)	+	104	0.41	-0.03	1.0	9	-
	Invasion year (n=21)	+	104	0.38	-0.02	1.0	9	-
Missing chela	*Intercept*	*NA*	*86*	-	-	-	-	-
	Distance from introduction (n=21)	+	86	0.25	0.05	1.3	9	-
	Invasion year (n=21)	+	86	0.28	0.05	1.4	9	-
Carapace length SD	Distance from introduction	-	57	0.15	0.10	1.0	9	-
	Invasion year	-	57	0.15	0.10	1.0	9	-
	Intercept	*NA*	*58*	-	-	-	-	-
	Crayfish density	-	58	0.15	0.09	1.0	2	-

2.11.4 Appendix 2.4: additional trends in rusty crayfish traits along their invasion gradient in the John Day River

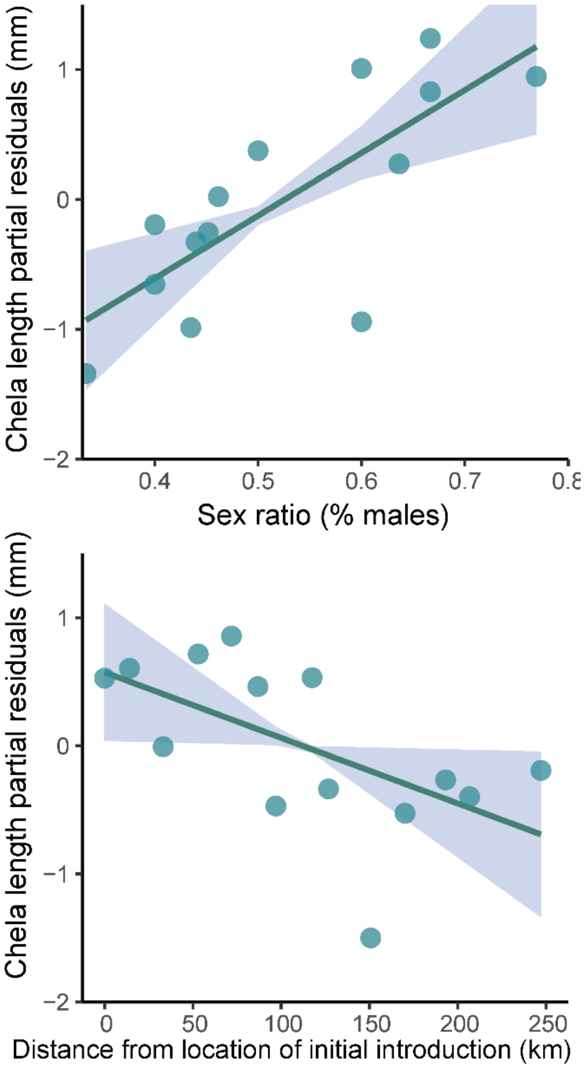

Figure 2.S2. Generalized Additive Model (GAM) predictions for rusty crayfish relative chela length (y-axis) as a function of the distance from their initial location of introduction (x-axis) in the mainstem of the John Day River. The smooth solid lines and shaded regions represent the predicted mean relative chela length and 95% Bayesian credible interval, respectively, from GAMs, while the points represent partial residuals.

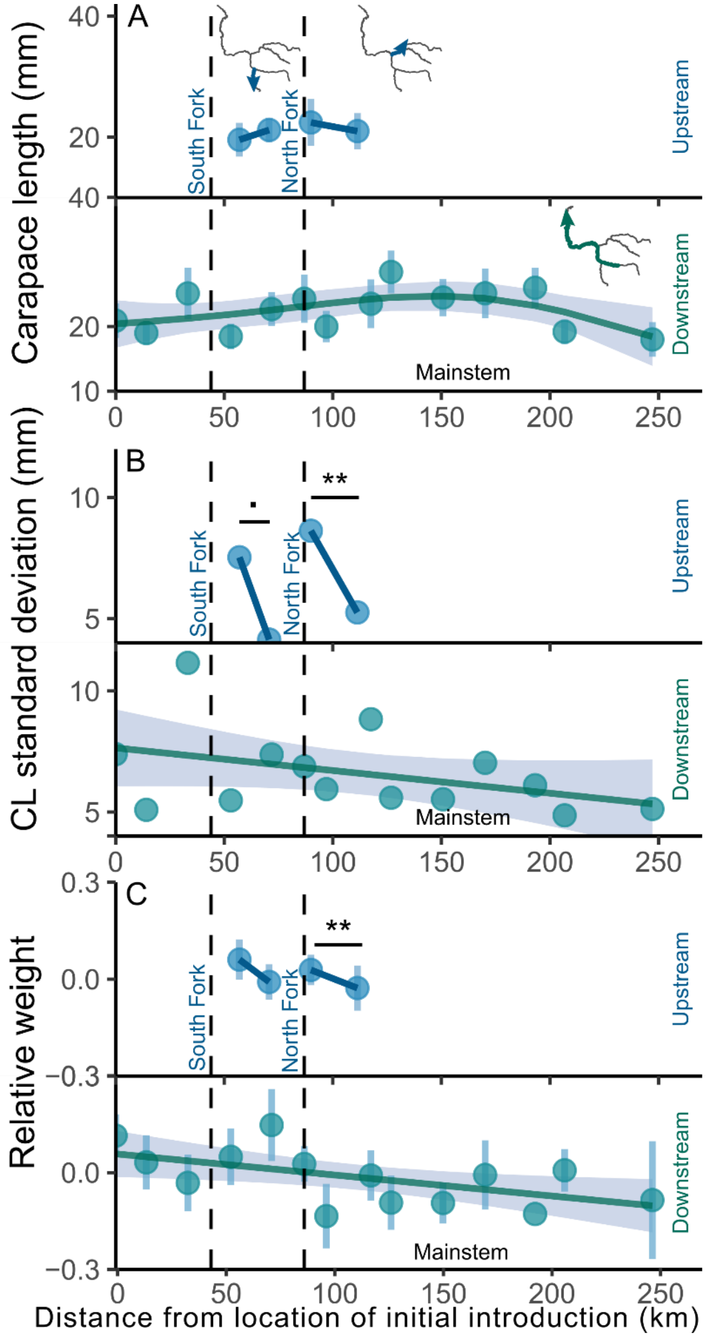

Figure 2.S3. Changes in mean crayfish relative carapace length (CL; A), carapace length standard deviation (B), and relative weight (C) along the invasion gradient of rusty crayfish in the JDR watershed. Consistent changes upstream (top panels) and downstream (bottom panels) suggest that differences in rusty crayfish traits stem from the range expansion itself rather than from longitudinal gradients in environmental conditions. The smooth solid line and shaded region represent the predicted mean trait value and 95% Bayesian credible interval, respectively, from a GAM, and asterisks denote significant differences in mean (‘***’: $p \leq 0.001$, ‘**’: $p \leq 0.01$). Vertical dashed lines represent the confluences of the South Fork (at 44km) and North Fork (at 87 km) with the mainstem JDR.

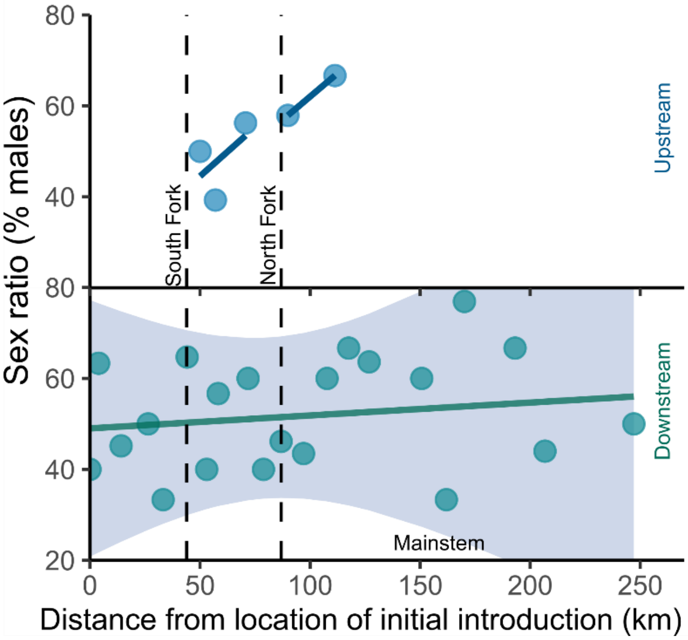

Figure 2.S4. Changes in the proportion of males in rusty crayfish populations along their invasion gradient in the JDR watershed. Consistent changes upstream (top panels) and downstream (bottom panels) suggest that differences in rusty crayfish traits stem from the range expansion itself rather than from longitudinal gradients in environmental conditions. The smooth solid line and shaded region represent the predicted mean sex ratio and 95% Bayesian credible interval, respectively, from a GAM, and asterisks denote significant differences in mean (‘***’: $p \leq 0.001$, ‘**’: $p \leq 0.01$). Vertical dashed lines represent the confluences of the South Fork (at 44km) and North Fork (at 87 km) with the mainstem JDR.

2.11.5 Appendix 2.5: temporal trend in relative chela length and weight of rusty crayfish from 2010 to 2016 at their initial location of introduction in the John Day River.

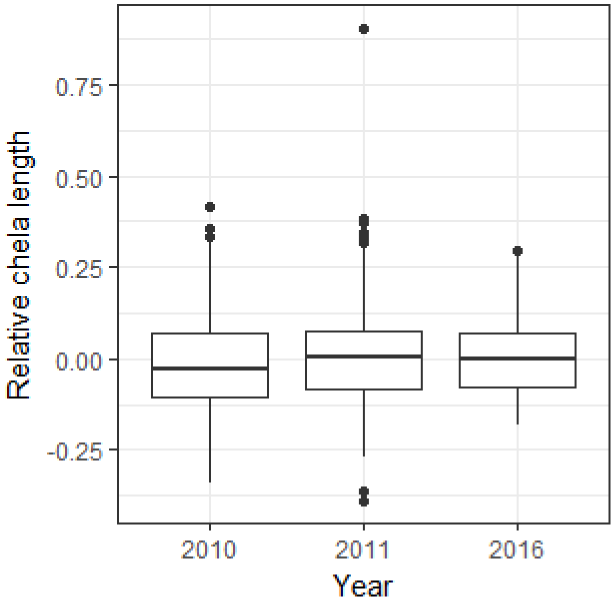

Figure 2.S5. Distribution of rusty crayfish relative chela length in 2010, 2011, and 2016 at their initial location of introduction in the John Day River. Relative chela length represents the residuals from a log-log linear least-square regression between carapace length and chela length using the pooled data for all years. Differences in relative chela length between all years were assessed for statistical significance using Welch's two sample t-test. Data are from Sorenson (2012) and this study.

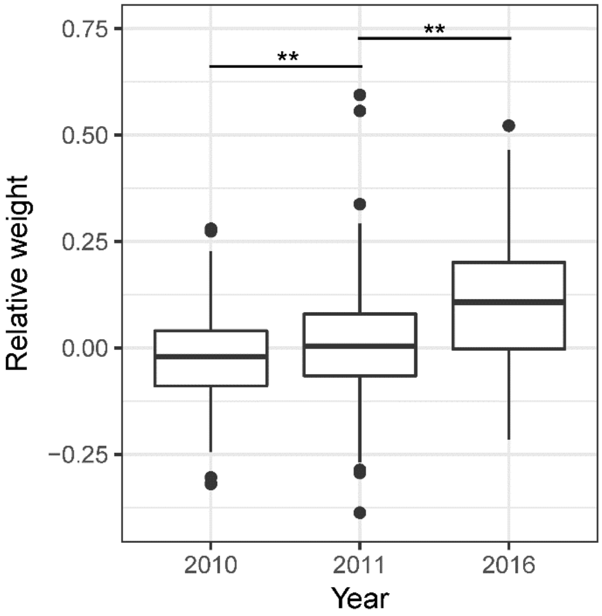

Figure 2.S6. Distribution of rusty crayfish relative weight in 2010, 2011, and 2016 at their initial location of introduction in the John Day River. Relative weight represents the residuals from a log-log linear least-square regression between carapace length and weight using the pooled data for all years. Differences in relative weight between all years were assessed for statistical significance using Welch's two sample t-test. Data are from Sorenson (2012) and this study.